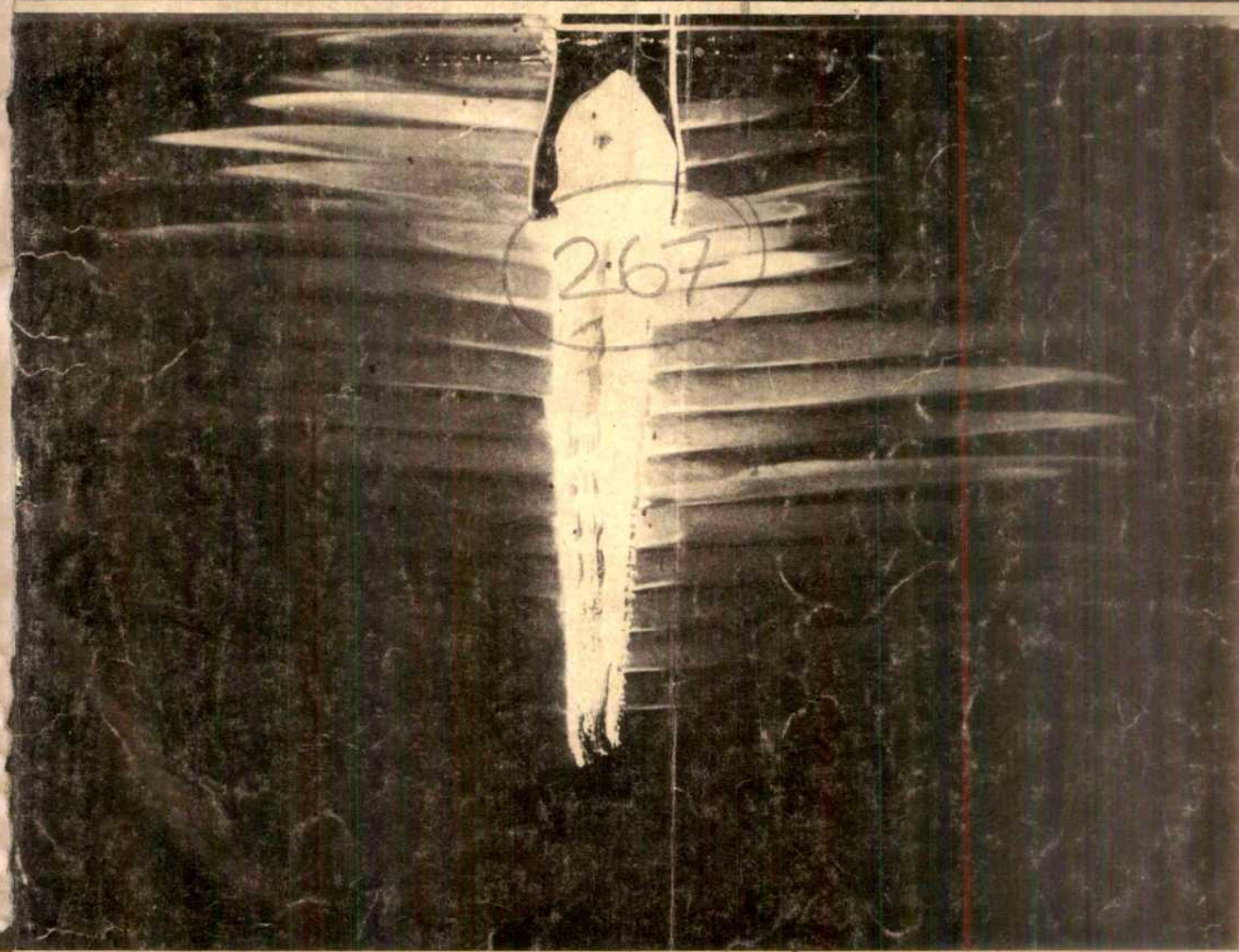


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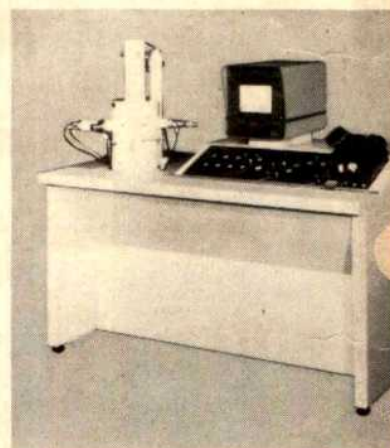
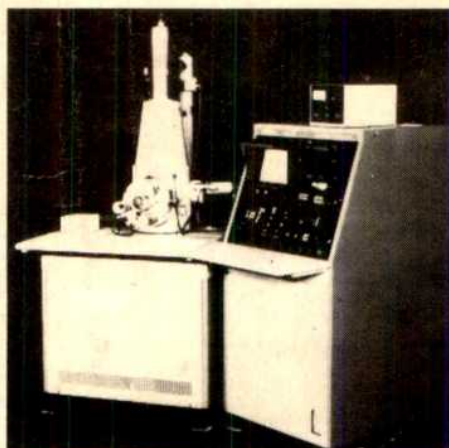
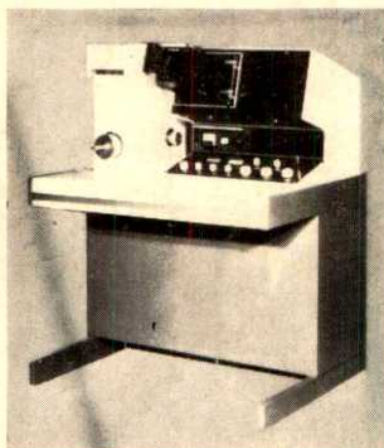
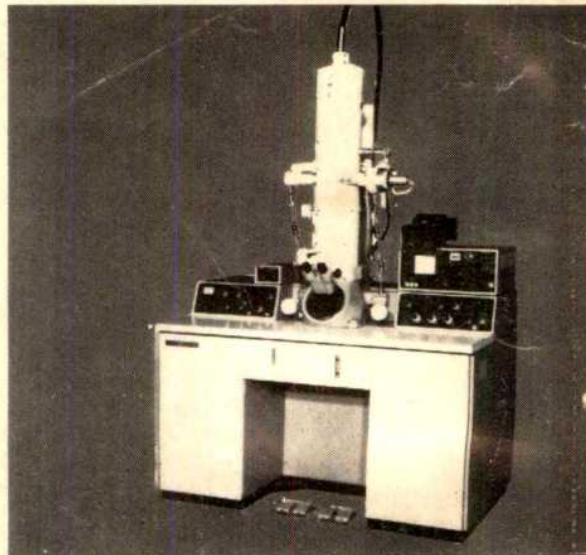
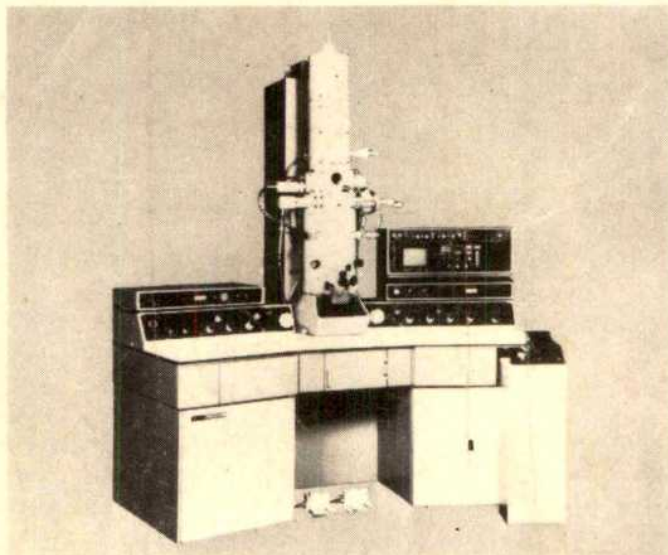
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© Macmillan Journals Ltd 1978
Published weekly
ISSN 0028-0836

Registered as a newspaper at the
British Post Office

London

4 Little Essex Street, WC2R 3LF
Telephone: (01) 836 6633 Telex: 262024
Telegrams: Phusis London WC2R 3LF

Washington

711 National Press Building, DC 20045
Telephone: (202) 737 2355
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Single copy (post-paid)
UK 80p Rest of world US\$2.50

Binders (post-paid)
UK £3.00 each, three for £8.00
Rest of world US\$6, three for US\$16

1976 Index (post-paid) U.K. £2.50. Rest of
World US\$5.00

Orders (with remittance) to:

Macmillan Journals Ltd, Brunel Road,
Basingstoke, Hants. RG21 2XS
Telephone: Basingstoke (0256) 29242

US Postmaster, please send form 3579 to Nature,
711 National Press Building, Washington DC 20045

Second-class postage paid at New York, NY
US mailing agent is:

Expeditors of the Printed Word Ltd.,
527 Madison Avenue
New York, NY 10022

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Shadowgraph photograph showing
tilted layers and interfaces produced
by a block of fluorescein-containing
ice melting in saltwater. See 'On
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nature

5 January 1978

UNCSTD—not just for bureaucrats

IN mid-1979 the United Nations will be convening a conference on science and technology for development (UNCSTD), probably in Vienna. It will be a large affair and can be seen as a successor to, and in some ways a consolidation of the earlier conferences on environment, population, food, women, habitat, water and deserts. Indeed those with long memories may recall that in 1963 the United Nations convened a similar large assembly in Geneva on 'the application of science and technology for the benefit of the less-developed areas'—a conference which left a very modest heritage. Many are wondering whether the 1979 conference will be any better than yet another forum for high-flown sentiments, thinly veiled abuse and meaningless resolutions. What happens in 1978, and particularly what scientists do for themselves will help to decide whether the meeting rises above the level of a circus.

UNCSTD is not about science and technology *per se*, it is about national and international policy on these matters. There are four expressed main objectives:

- to decide on ways and means to apply science and technology in establishing a new international economic order,
- to strengthen the technological capacity of developing countries to enable them to apply science and technology to their own development,
- to realise scientific and technological potential in solving development problems of national, regional and global significance, especially for the benefit of developing countries, and
- to provide instruments of cooperation to developing countries in the use of science and technology for solving socio-economic problems that cannot be solved by individual action.

Talk of a 'new international economic order' does not, of course, exactly commend the conference to those developed nations which are suspicious of this concept;

even so, they, along with developing nations, have received a detailed request for the submission of national papers relevant to the conference and it is from these papers, together with the proceedings of regional conferences now being held, that the detailed agenda for UNCSTD will be forged.

The value of such a conference is undoubtedly that it causes some important people in government to have to stop and think about policy or lack of it. In this way the application of science and technology for development might no longer be regarded as a bit of an insignificant backwater in the developed world or as something which has to undergo violent changes in its level of support in the developing world.

The weakness of UNCSTD, however—or at least its potential weakness—is that scientists and technologists may not get a look in; the conference could be entirely bureaucrats talking to bureaucrats. It is possible for this to happen; the conference's secretary-general, Joao da Costa, a Brazilian scientific diplomat, fully admits in a recent issue of *Development Forum* that the secretariat has no authority to bypass governments and go directly to the masses—all it can do is encourage governments to hold discussions with scientists, trade unions, professional organisations and so on. Thus the voice of the practitioner need only be heard if the government chooses to ask for it.

There is, therefore, a need this coming year for scientists and technologists to speak up and use every channel that is open to them to tell their colleagues, in their own countries and around the world, how science and technology is being used and how they think it should be used for development. This was an appeal made very clearly and eloquently in *Nature's* columns recently by Michael Moravcsik of the University of Oregon (24 November, page 228) and we hope that his message will encourage more people to toss their ideas into the arena. Certainly *Nature* will try to be receptive over this coming year to those in the developing world who wish to speak to a large audience. □

Out of the frying-pan into the PWR

Amory B. Lovins, a consultant physicist, and British Representative of Friends of the Earth Inc., the US sister-group of the independent UK group FOE Ltd., argues that US pressurised water reactors are an economic disaster

THE juggernaut advance of light-water reactors (LWRs), and especially pressurised-water reactors (PWRs), through world nuclear markets has seemed to their promoters a sort of manifest destiny. When Britain rejected PWRs for the second time, in 1974, the president of Westinghouse, which first developed them, replied magisterially that this was only "an interim solution, as we are not convinced that it addresses Britain's long-range energy needs." Since then, British electricity use has risen by less than 2%, while excess output capacity above a reasonable 20% reserve margin has risen to 20% of peak output and may soon be 30 to 45%. Closer analysis of energy use has also revealed not only that far more efficient use and even proven renewable sources are practicable and advantageous, but also that Britain already has twice as much electricity as is needed for the premium uses that can give value for money from this very costly, high-quality form of energy. Yet Westinghouse and its allies—including European vendors, the National Nuclear Corporation (NNC), and the Central Electricity Generating Board (CEGB)—are now back again. They argue that the British preference for advanced gas-cooled reactors—the last major indigenous programme of thermal reactors outside Canada—is a sentimental anomaly that now, bereft of further excuses, must give way to technical and economic rationality.

In an open letter to the Prime Minister of the United Kingdom, James Callaghan, on 21 December, Friends of the Earth document a counter-argument. They suggest that the worldwide dominance of PWRs has arisen not from merit but from aggressive salesmanship and "a process of 'mutual intoxication' whereby US promotional institutions persuaded each other, then their counterparts abroad, that LWRs' supposed merits were real and had been demonstrated when in fact they had not and still have not been realised: the distinction between promotional prospectus and critical evaluation was completely obscured". Thus, according to a forthcoming study*, the gas-graphite system displaced in France by PWRs was in retrospect superior, but policy-makers were caught up in a skilfully propagated and wholly unfounded PWR euphoria. Systematically mistaking hope for fact, they thought they *knew* how much PWRs would cost to build and to run, how reliable they would be, and what fuel burnup they would attain. US experience has fallen far short of these expectations. Real capital costs have averaged more than twice as high as promised; real fuel-cycle costs will be about six times as high. Capacity factor has averaged 20 to 30% lower and burnup 20 to 40% lower than forecast. The picture is not getting brighter. Two recent studies, for example, show PWR capital costs rising by 20% y^{-1} or \$188 $kW(e)^{-1} y^{-1}$ in constant 1976 dollars—about three times as fast as coal-fired stations. Much European and Japanese experience is similarly disappointing. Yet NNC's latest report on reactor choice is not an analysis of independently established facts so much as a seller's advertisement of alleged virtues. It is remarkably like the promotional paper that USAEC staffers drafted for the European Community's "Three Wise Men" when they recommended twenty years ago that Europe switch to LWRs.

*Light Water: How the Nuclear Dream Dissolved (New York: Basic Books, March 1978), by Professor I. C. Bupp (Harvard Business School) and Dr J.-C. Derian (an official of the French regional development ministry DATAR).

In the US, the euphoria has worn off abruptly. The doubtful economics of real (as opposed to paper) PWRs, uncertainties about demand, reliability, and safety, and the macroeconomic problems of a capital intensity 10 to 30 times that of new North Sea oil capacity have together led to what FOE call "the most dramatic collapse of a major industrial enterprise in history". US yearly domestic orders in 1972–6 (net of cancellations ascribed to the year of original ordering) were respectively 28, 38, 17, 5, and 3 reactors. In 1975–6, seven reactors were ordered, eight cancelled, and 13 deferred (six indefinitely). In 1977, two were ordered, four cancelled, and 26 deferred. This quickening disintegration has led Dr Schlesinger's deputy to state that "the nuclear option has essentially disappeared" in the US.

Official forecasts of US nuclear capacity in the year 2000 have been falling so quickly that, extrapolating linearly, the 1978 forecast for 2000 should be zero (in fact the asymptote might be as high as 24% of present US delivered energy use, or about the level of firewood). Nuclear expectations are likewise plunging in France, Germany, the UK, Japan, and elsewhere at such a rate that the 1979 forecast of 1985 OECD nuclear capacity should be zero. In Canada, which has had none of the US regulatory problems, forecasts have dived just as steeply as in the US, suggesting that the cause is not some political artifact but fundamental market forces—which President Carter's energy policy does nothing to discourage and much to reinforce. In short, PWRs are proving all but unsaleable throughout the industrial world. Staunching the rapid haemorrhage of money and staff from reactor vendors is requiring proliferative exports to developing countries, lavishly subsidised by exporting governments. This is not much of a vote of confidence in PWRs or in Britain prospects for profitably exporting them. Indeed, reports in the financial press suggest that no LWR vendor has sold reactors at a profit: vendors' cumulative losses are said to exceed £1 000 million in the US (over half of it for Westinghouse alone), £250 million in Japan, over £200 million in West Germany, and large but undisclosed sums in France and elsewhere. This hardly seems a promising line for Britain to follow—salvaging and refloating other countries' lame ducks.

Further, while PWR advocates consider the vexed question of pressure-vessel rupture adequately resolved, FOE (like most US observers) have long put this issue rather far down a very long list of serious safety problems. Their letter documents ten compendia showing over 200 unresolved major safety problems of PWRs, most of them officially acknowledged. British advocates' optimism about PWR safety may rest not on the detailed knowledge which they claim, but on the lack of it which they have in the past displayed (notably in the 1973–4 controversy) and which the uncharitable can infer from the 1977 NNC report. It is possible that the NNC, CEGB, DOE, and Nuclear Inspectorate staff have in fact all done their home work. There is, however, no published reason to believe this is the case. They may still not have consulted the main original sources, preferring to rely, as in 1973–4, on summaries prepared by US parties that could hardly be considered disinterested. FOE have therefore proposed that specified papers well known in the US debate be the subject of public colloquy in Britain between highly qualified US critics and their British adversaries.

Only four years ago, the NNC and CEGB were pressing for an urgent programme of 32 PWRs—which, if adopted, might well by now have pushed the British nuclear industry over the brink of ruin. The advocates of haste that time were wrong; the advocates of caution were right. So it may be again.

German leader for JET project

Dr Hans-Otto Wüster, a West German accelerator physicist presently on the directorate of CERN, was named on 20 December as project leader for the building of JET, Europe's planned giant torus for extending fusion energy research. Dr Paul Rebut, the French physicist who has led the design team for JET, has been named deputy, and Dr Romano Toschi (currently head of the Italian fusion effort based at Frascati) will be Chairman of the Management Committee. All three appointments are provisional, but are expected to be adopted formally by the Council of Ministers early in 1978.

Dr Wüster's appointment is bound to be felt as somewhat of a rebuff to Rebut: but it was not unexpected. This is not because of any failing in Rebut, but because of the vexed European politics that have dogged JET from its inception. When the decision was made last year that JET should come to the Culham fusion laboratory in the UK (where Rebut's design team had been based) rather than to Garching in West Germany, it was felt that some kind of recompense should be paid to Germany. The appointment of Wüster to the top job achieves this—if to the chagrin of France.

Wüster is not well known at Culham—his field has been high energy physics, not fusion—and he

may find his initial reception cool. But he has time yet to work out his relationship with Rebut and Toschi and those of the design team still left at Culham. The Interim JET Council that appointed him, composed of representatives of all the nations contributing to JET, is suffering from the usual problems of large international committees and is not making its decisions particularly rapidly. It seems unlikely that the team will be completed before July, and meanwhile the main competitor—The Princeton Tokamak—has a chance to forge ahead. After all the earlier delays in the JET project Culham staff look on this further constraint somewhat resignedly.

Wüster himself is no stranger to large international scientific projects. He was deputy to John Adams during the building of the 400 GeV SPS accelerator. Once it was agreed to build it, the SPS became a great European success—built under budget, ahead of schedule, and beyond specifications. Wüster managed the budget of the SPS, and no doubt his success there influenced the JET Council. It will be interesting to see if he can be successful with the budget and management of JET, in a technology he knows less well.

Staff at CERN respect Wüster as a man with great administrative ability, a man who has his goals very clearly



Hans-Otto Wüster: goals clearly in mind

in mind, a blustering, jovial man "who will pile in rapidly" and take to his job with enthusiasm.

Another undoubted asset Wüster will bring to JET is his long-standing friendship with his opposite number at Princeton: Dr Paul Reardon who heads the Princeton Tokamak team. Reardon was also a high energy physicist; and he played a similar role to Wüster's in the building of the Fermilab National Accelerator, the 400 GeV US competitor to Europe's SPS. Fermilab was built first, and skimmed the cream, because of the delays that hit CERN in finding a site and funding in Europe. Wüster will no doubt go all out to prove that Europe can, sometimes, be first.

Robert Walgate

Construction shares

ONE of the most pressing tasks the UK research councils will have to tackle early in the new year will be to decide how to spend their individual allocations of the money promised them late last November and early December for construction and energy conservation. Firm decisions must be made very soon because the money has to be spent in 1978-79.

The Advisory Board for the Research Councils (ABRC) announced its decision on how to divide the total sum between the individual research councils just before Christmas: a total of £4.5 million had to be shared between four research councils (the Social Science Research Council did not put in a bid) and the British Museum (Natural History). Initially, £4 million had been set aside for the recipient bodies of the science budget to spend on construction during 1978-79; but in December, when the Secretary of State for Energy announced the UK's £320 million energy conservation programme, the sum was increased to £4.5 million. £1.1 million of it is to

be spent on energy conservation.

The £4.5 million has been divided as follows: the Agricultural Research Council is to receive £1.07 million, of which £0.27 million is for energy conservation; the Medical Research Council, £0.73 million, of which £0.13 million is for energy conservation; the Natural Environment Research Council, £0.65 million, of which £0.15 million is for energy conservation; the Science Research Council £1.75 million; and the British Museum (Natural History) £0.3 million.

The Natural History Museum probably has the firmest plans of what to do with its share. Most of it will be used to provide accommodation for fossil collections and staff at the museum's station at South Ruislip whilst preparations for a new exhibition hall in part of the main building get underway.

As yet, however, the research councils claim to have few firm plans for spending their shares, even though they must each have put forward definite proposals to the ABRC. But they do agree that major new capital projects are out of the question:

the fund is too small and the time available too short. Most of the money will be spent on extending or improving existing buildings or speeding up construction work already started. For example, the SRC will use some of its share to speed up work on the nuclear structure facility at the Daresbury Laboratory and to extend some of its central user facilities. The NERC may use some of its share to put into operation existing plans to extend facilities at the Institute of Hydrology.

Judy Redfearn

BBC commemorates Bronowski

GEORGE Steiner, a controversial man of letters and champion of science, who learned physics from Enrico Fermi and chemistry from Harold Urey, will broadcast live in the UK on BBC2 television next Tuesday at 8.10 pm in the first Jacob Bronowski memorial lecture. Bronowski, devoted to what he called 'the democracy of the intellect', literally gave himself to an epic tele-

vision series—"The Ascent of Man"—which presented his personal and heroic view of science as the saviour of mankind. Bronowski died shortly after completing the programme; these lectures are the BBC's tribute to him.

Steiner is an interesting choice to lead off the memorial lectures. He has many points of contact with Bronowski; and as many differences. Both were Jewish exiles from Europe: the Nazi holocaust is writ large in both their lives, and both have shown themselves aching to find escape from it. The ultimate intellectual sanctity of science, with its references to verifiable fact, particularly in the physical sciences and mathematics, appealed deeply to both men as the antithesis of dogmatism. But whereas Bronowski searched for a release, in the fashion of The Enlightenment, for all of mankind, Steiner appears to look only towards an elite, a bright spark in the

darkening eclipse of humanity.

Steiner will argue in his lecture that it is pointless to look for controls on the pursuit of science; for knowledge will out, somewhere, sometime. One day scientists will make their test-tube babies. One day there will be genetic engineering of human beings. We are powerless against the march of truth. Steiner will prove himself the hawk of science, where Bronowski was the dove. In the process he may do science a disservice. He is a great acquirer of knowledge: "What else is there?" he asks. Bronowski also asked for compassion, an emotion that Steiner might be suspected of dismissing as romantic.

In the 'Ascent of Man' Bronowski drew attention to a man who he said was the most intelligent he'd known: Johnny von Neumann, the originator of games theory. Von Neumann failed mankind, said Bronowski, as an intellectual elitist who believed

not in the democracy but the aristocracy of the intellect. Steiner appears to fall into the same mould as von Neumann.

Steiner learned science at the University of Chicago in the late 1940s. He "scraped" a first and to his lasting regret was turned away from science by his careers advisor who said he could not cope with physics (but that was Chicago in the days of Fermi; Lee and Yang were students) and that the rest of science was "bottle-washing". Steiner, fluent in four languages, turned to journalism and ultimately to the study of language.

He would have liked to have been a biologist. His passion is mathematics and his heroes are mathematicians. The creation of melody, and of proofs (in mathematics) that the truth of a certain proposition is undecidable, are, he believes, the greatest mysteries.

Robert Walgate

ESA names European candidates for first Spacelab mission

THE European Space Agency (ESA) has named the four candidates from whom one will be selected to serve, along with one American scientist, as a "payload specialist" on the joint US-European Spacelab mission scheduled for the second half of 1980. The candidates were presented at a press conference on 22 December at ESA's headquarters in Paris. They are Franco Malerba, 31, an Italian electronics engineer and physicist; Ulf Merbold, 36, a West German physicist and staff member of the Max Planck Institut für Metallforschung in Stuttgart, where he has been researching crystal lattice defects; Claude Nicollier, 33, a Swiss astronomer; and Wubbo Ockels, 31, a Dutch physicist who is doing research at the Nuclear Physics Accelerator Institute in Groningen.

With the exception of Malerba, all are married with one child. Ockels is the only non-flier in the group. Merbold is a glider pilot, Malerba holds a private pilot licence and Nicollier, after working as a professional pilot for Swiss Air, is still in the Swiss Air Force.

After three months of Spacelab familiarisation at European facilities and further evaluation in Houston, one of the four candidates will be eliminated. After next May the other three will continue training until a few months before the flight when one of them will be chosen to be the first European to travel and work in earth orbit. The other two will act as back-up specialists on the ground. On the first ESA/NASA mission lasting one week, the two scientists will be responsible for a payload of 76 experiments in the fields of materials science, atmospheric physics, life sciences, solar physics,

astronomy, earth observation and space technology.

"We were not looking for a superman or superwoman," said Michel Bignier, Director of the Spacelab programme. He explained that the scientists would be working in a pressurised "shirt-sleeve" atmosphere. Nonetheless, the evaluation criteria had been particularly severe—equivalent to level II criteria used by NASA to select permanent mission specialists. "Nobody wanted to run the risk of a rejection by NASA," added Mr Bignier.

By last September, 53 candidates had been preselected from 2,000, including 35 women, who had responded to ESA's announcement of opportunity issued in April. One woman, Anny-Chantal Levasseur—regourd of France, was among 12 finalists but failed on the last medical test.

Candidates were screened by four panels of experts covering general

assessment and system engineering, scientific, psychological, and medical tests. All the candidates agreed that the psychological testing, carried out at the DFVLR Institute for Flight Medicine in Hamburg, was particularly arduous. Klaus-Martin Goeters, a member of the institute and head of the psychological panel said that tests were aimed at ensuring that those selected could cope with the stress created by the heavy Spacelab workload and the unusual environment. Drawing a typical personality profile, Dr Goeters described the successful candidate as "someone who is highly motivated, with a tendency to be an introvert, preferring small groups to a larger social context, who is unpretentious in habits and life style, thus capable of envisaging certain privations, and is highly mobile: that is a person interested in and looking for new things—with, you might even say, a taste for adventure".

Betty Werther



Claude Nicollier, Ulf Merbold, Wubbo Ockels, Franco Malerba: Europe's spaceman scientists

GMAG: Stormy weather ahead?

1978 may prove to be a difficult year for Britain's Genetic Manipulation Advisory Group. David Dickson reports

GMAG, which was set up by the UK Government following concern at the implications of research into the genetic manipulation of micro-organisms, celebrated its first birthday last month, thereby reaching the half-way stage of its first term of office. So far both scientists and trade unionists have given it, in general, a clean bill of health; comments on its performance range from "as good as could be expected" through "better than anticipated" to "a highly successful experiment".

Applications for more than 80 experiments, from about 25 different institutions, have been received, and all have been approved. According to Sir Gordon Wolstenholme, director of the CIBA Foundation and chairman of GMAG, the first year has been "surprisingly profitable and useful".

Yet if the first year has been relatively calm, there are storm clouds on the horizon. Two factors in particular are likely to put pressure on GMAG's activities in the near future, and to test its skill in treading a delicate path between different—and sometimes opposing—interest groups.

The first of these factors is industry's rapidly growing awareness of the financial rewards offered by genetic engineering techniques, from the production of artificial hormones to, possibly, new plant crops. Already seven major US companies have established recombinant DNA research laboratories, and several British companies, after a relatively slow start, are jumping on the band-wagon.

The second factor is the additional knowledge, both about genetic structures and the potential hazards of their manipulation, that has emerged in the four years since a temporary moratorium was first suggested by US scientists. This knowledge suggests that some of the initial reaction was over-cautious, and that current guidelines might be "safely" relaxed.

Of these two, it is the industrial applications that have so far impinged most directly on GMAG's activities, primarily through the issues of confidentiality. For genetic engineering is rapidly becoming a field in which the dividing line between science and commerce is frequently indistinguishable.

In the US, for example, shortly after a team of San Francisco scientists headed by Dr Herbert Boyer confirmed that it had successfully inserted into bacteria a gene capable of producing the hormone somatostatin, a small Californian company Genentech an-

nounced that it would be using the technique to produce somatostatin for sale to research organisations and pharmaceutical companies by mid-summer 1978, at a cost considerably below the current market price.

In Britain, the National Research and Development Corporation—the body responsible for patenting and exploiting the results of government-sponsored research—recently held a lively and well-attended meeting for university scientists on the potential applications of genetic engineering. NRDC has already awarded a research contract to the John Innes Institute near Norwich for the study of improvements in the production of the antibiotic streptomycin, and other projects are in the pipeline.

Although some companies, such as ICI, have been studying the applications of genetic engineering techniques for a number of years, little is publicly known about how far such work has progressed. For with the potential rewards so enormous, not only commercial companies, but all those with potentially-patentable techniques, are keen to play their cards as close as possible.

There is therefore an inevitable reluctance to reveal even in outline details of future research programmes to a group such as GMAG over whose use of the information—notwithstanding the Official Secrets Act—an individual company can exercise no direct sanction.

The confidentiality issue is one which the difficulty of reconciling "health and safety" factors against "national interest" has provided GMAG with some uncomfortable moments. In particular, the four trade union representatives—two from the Association of Scientific, Technical and Managerial Staffs (ASTMS), one from the Institute of

Professional Civil Servants, and the medical officer of the Trade Union Congress—while respecting the need to maintain confidentiality for commercial reasons, have refused to be bound by any secrecy agreement which might prevent them from serving the interests of their members.

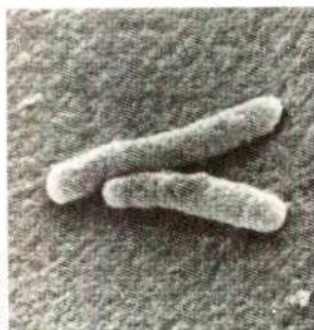
Many hours of discussion have been spent trying to devise a formula by which secrecy can be guaranteed for research programmes which must soon, under new health and safety legislation, be reported to the Health and Safety Commission. Eventually a compromise formula has been agreed under which, for a trial period of six months, members of GMAG will agree to a simple secrecy pledge, and applications for commercially-sensitive experiments will be seen by all except those considered to have some direct interest.

But for most scientists, the confidentiality issue, apart from interfering with claims for scientific precedence, remains largely peripheral. Of far greater concern is the type of pressure that may be put on those responsible for maintaining safety guidelines as a result of industry's interest.

In particular, many fear that industry's desire to pursue and exploit the applications of such research as quickly as possible may result either in pressure on governments to reduce containment requirements too far, or to remove them altogether, leaving the management of risk to the vagaries of the market place (and the accountancy skills of insurance companies).

There are already pressures from scientists themselves to rationalise present containment levels, bringing them more in line with procedures adopted for other types of experiment using known pathogens, and taking into account both the development of new genetic engineering techniques and of more precise assessments of hazards.

Research by Professor Roy Curtiss at the University of Alabama in the US, for example, has confirmed that the K-12 strain of *Escherichia coli*



Above: view of *E. coli* by scanning electron microscope; right: researchers working in a high containment laboratory



NIN

most commonly used in present recombinant DNA research is unable to survive in the human intestine like normal strains, and is thus very unlikely to cause an epidemic if it escaped from the laboratory.

This research has also led to the development of a "disabled" strain X-1776 which has already been passed by GMAG for some high-risk experiments. And it is widely expected that the successful development of a host-vector system using another "disabled" strain of *E. coli* with equally "safe" properties will be announced soon in the UK.

Such developments are already encouraging a certain relaxation of physical containment levels, in particular since the greater the biological safety of the organism, the lower the necessary degree of containment. In the US, for example, the National Institutes of Health published last summer proposed revisions to the guidelines first issued in June 1976. The new draft guidelines, which were publicly debated in Washington just before Christmas, and have already aroused considerable opposition from environmentalist groups such as Friends of the Earth, involve a considerable reduction in the containment levels for certain widely-used experiments.

Perhaps the most controversial proposal is to reduce the required physical containment level for experiments using recombinant DNA from mammals other than primates from P3 to P2; in practice this means that experiments using, say, mouse DNA which under the current regulations require purpose-built facilities, could be carried out on an open laboratory bench with a few relatively minor adaptations.

At present it is uncertain whether this particular proposal will be accepted (although others, such as a revised system defining physical containment requirements, probably will). Certainly many British scientists feel that, in the present state of knowledge, this may be relaxing things a little too far, at least for "shot-gun" experiments using unpurified DNA.

Yet the main concern about proposed revised guidelines is not so much their scientific content, but the fact that, if adopted, serious discrepancy between British and American laboratory practices could result, with potentially embarrassing consequences.

It could, for example, be cheaper to send a research worker to the US to carry out a set of experiments requiring little more than a conventionally-equipped laboratory bench, than to instal—or even hire—facilities providing the higher physical containment levels required to carry out the same experiment in the UK.

Such embarrassment is likely to be reinforced if other European countries, most of which have so far followed the British guidelines as suggested by the Williams Committee in 1976, decide to break ranks and, in line with the US, introduce significantly lower containment levels (as the French are, indeed, now proposing to do).

If, for example, Germany decides to introduce less stringent guidelines, British scientists working at the European Molecular Biology Laboratory at Heidelberg, which operates under German laws, may find they are permitted to carry out experiments which they are unable to do at home.

In such a situation, GMAG could find itself caught in a more difficult dilemma than that which it has already faced over confidentiality. For while responding—reasonably—to demands from scientists to rationalise containment levels, public confidence requires that it must not be seen to be responding to outside, and particularly industrial, pressure.

The members of GMAG are conscious that if they impose too harsh a set of restrictions on industrial research programmes as compared with other countries, companies will merely transfer their research programmes—and their revenue-earning potential—elsewhere; "and we don't want a repeat of the penicillin story", according to one GMAG member.

However, the Williams Report, which itself followed the public discussions generated by the Ashby report of 1975 and received evidence from a wide range of bodies, is still less than two years old. Any major attempt to re-draft its proposals at this stage is likely to be seen as premature, if not by scientists at least by the public, to whom some of the more fanciful possibilities (and dangers) of genetic engineering have only recently come home.

Indeed, it is one of GMAG's strengths that, through the trade union and "lay" representatives, it is able to take public concern directly into consideration (thus avoiding the major confrontations between scientists and the public that have, for example, occurred in the US).

Yet as the pressures on GMAG increase, so the tensions between its constituent parts may become more difficult to control. Such tensions arise partly from the ambiguous nature of GMAG itself, seen by some as an unfortunate, if necessary, part of scientific bureaucracy, and by others—including the trade unionists—as a successful model of public participation in research policy, with potential applications in other areas.

Disagreement over how to handle the confidentiality issue has already

brought these tensions to the surface. And while there is little criticism of the behaviour of the trade union representatives on scientific aspects of GMAG's work, they have already been accused of trying to make "political" points out of issues such as the future of the Ministry of Defence's Microbiological Research Establishment at Porton Down (part of which is likely to be transferred to the Department of Health and Social Security for carrying out top risk experiments requiring Category IV containment levels).

The political temperature of the debate has been raised by the recent decision of the British Society for Social Responsibility in Science to set up a genetic engineering group. This has already attacked the "complacent" attitudes of many scientists towards the safety aspects of such work. And the BSSRS group also feels that safety guidelines should have the force of law.

These issues will also undoubtedly be aired at the public hearings into genetic engineering soon to be held by the House of Commons Select Committee on Science and Technology. Reactions to the decision to organise these hearings vary; some are wary of the effects that renewed public exposure will have, and of possible "anti-scientific feeling" on the committee, while the BSSRS group has, in contrast, given the decision enthusiastic support.

But there are a number of important issues in need of attention. One of these is the make-up of GMAG itself, whose members are due to be reappointed at the end of 1978. Many would like to see more scientists on the group, particularly experts in fields of research not adequately covered at the moment. And there is still a residual unhappiness that although GMAG has two ASTMS representatives, there is no member of the Association of University Teachers acting in a representative capacity.

Another issue to which the Select Committee may give its attention is the intensive, quantitative study of the potential hazards of genetic engineering research. So far, despite an apparent commitment both in the US and the UK to the need for such work, little has yet been done by the scientific community actively to promote it.

GMAG is due to present its first annual report to Mrs Shirley Williams, Secretary of State for Education and Science, in a few weeks time. One thing is already clear: that GMAG's role is likely to become both more important and more difficult. As Sir Gordon says: "If anything, our 'political' problems are not going to die out, they will increase; and not because anything is going wrong, but because it is in the nature of the animal." □

correspondence

Austrian science journalists were warned

SIR,—As one of the people principally involved in disclosing the fraudulent activities of Schaden ('Austrian science minister attacks science journalists in fraud case' 24 November, page 292), I should like to point out that in 1973 I warned Austrian scientists and science journalists about the questionable 'discoveries' of Schaden and Celta. Unfortunately, as the Science Ministry's document demonstrates, this was to little avail.

The purpose of the document and the Ministry's previous interventions and warnings, was to protect the scientific community from public discredit on account of a charlatan's 'research'. It was also to serve as a constructive warning to scientific journalists not to take every apparent scientific 'break-through' at its face value.

If the Science Ministry's document lacks thoroughness, this will certainly be supplied by the court's records of the Schaden case.

Yours faithfully,

WILHELM GRIMBURG

Vienna, Austria

A third world energy view

SIR,—Effective use of the world's finite cheap oil reserves to save time and effort deserves a broader discussion than that given by Alvin M. Weinberg (20 October, page 638). Efficiency of the conversion of energy into time saved is highly variable. To travel 15 miles, for large numbers of people, means walking in the tropical heat carrying luggage and taking, say 5 hours. To be lucky enough to have a small motorcycle (and do the journey in 30 minutes on 0.1 gallons of fuel) saves 45 gruelling man hours per gallon. To then change to a large car (and come back at 90 miles per hour using 1 gallon of fuel) will only save a further 0.37 man hours per gallon.

The assertion that "a free society allows each of us to make the choice" and "economics . . . integrates all these . . . judgments" means that the rich have the right to decide. But when availability of cheap oil begins to decrease these judgments will, no doubt, be re-evaluated, and those who make them now thus carry a heavy respon-

sibility. Surely they will be expected to justify their judgments when others reach the position where they could use oil but find that it has all been carelessly squandered to save milliseconds of millionaire's time per gallon.

Yours faithfully,

M. D. MELAMED

Maputo, Mozambique

Desert rainfall

SIR,—J. L. Deaton (24 November, page 294) makes several statements concerning our article (19 May, page 192) with which we disagree. He claims, in particular, that "in no sense is the mean too large". There is, however, a well defined sense in which the mean of a positively skewed distribution (for example, precipitation) is "too large". Specifically, it may be desired that a statistic, to be taken as a measure of central tendency, has the property that either it represents the midpoint of the distribution (that is the median), or that it represents the value of the distribution which is most likely to occur (that is the mode). If the distribution is positively skewed, then the mean is larger than the values satisfying either of these properties.

Deaton also claims that the mean "is quite indicative of how much rain commonly falls in most regions—even Gao and Niamey." But the mean, at least in some sense, is not at all indicative of how much rain "commonly" falls in the Sahel. As an example, consider the Gao August precipitation data used in our article: only 4 of the 35 observations (11%) fall within 10% of the mean; 23 of the 35 observations (66%) fall below the mean; and the mode (or most 'common' value) is 80.0 mm, substantially less than the mean of 100.9 mm.

Deaton states that "there seems to be only a slight tendency for the degree of skewness to increase as the average amount of precipitation decreases". This statement contradicts the results of studies on this issue in the climatological literature (Arnold Court in *Climates of North America*. World Survey of Climatology 11, 212 (Elsevier, Amsterdam 1974)). While it is true that all precipitation distributions are at least slightly positively skewed, there is a marked tendency for the

degree of skewness to increase as the mean precipitation decreases.

Finally, Deaton asks for documentation of the observation that "recent weather tends to influence perceptions more heavily than earlier weather and wet spells more heavily than dry ones". The documentation for this statement is a quote from J. C. Caldwell "Rain-fall Statistics, Droughts, and Desertification in the Sahel," *Desertification*, 84 (Westview Press, Boulder, Colorado 1977).

Yours faithfully,

MICHAEL H. GLANTZ

RICHARD W. KATZ

National Center for
Atmospheric Research
Boulder, Colorado USA

IQ or intelligence type?

SIR,—The controversy over race and IQ is clouded by the lack of culturally neutral testing procedures. Who would we be to say that a community of blacks who tested slightly lower than comparable whites were not immeasurably superior in forms of mental ability not emphasised in the test procedures? A slight mean shift from one community to another would doubtless be given more weight than it deserves, and it would still be possible (if its Gaussian distribution was shallower) for the 'less endowed' community to provide more geniuses than its counterpart. Furthermore, the difference between the two means would certainly be less than that between any two members of either group that you might encounter in the street, but would this be taken into account by those with political motivations? Data may be used to substantiate whatever case and in an area like this the phenomenon could have serious consequences. The "compensatory advantages" Sir Andrew Huxley cites in his address to the British Association annual meeting would soon be seen as racially-discriminative favouritism, and is it really so likely that humane considerations would moderate the debate? We take scientific findings much less seriously than it is popular to imagine: the new evidence in fields such as psychokinesis, spoon-bending, E.S.P., and tobacco smoking has done little to alter the attitudes of those with convictions of convenience that contradict the specialist conclusions, and there are still

eminent scientists who refuse to accept evolutionary theory and rely instead on more metaphysical alternatives. Scientific findings do not alter human belief so readily.

Huxley says that it is a characteristic of research that its outcome is not known in advance. That is so: but it is a presupposition of outcome that often motivates research, which may bias its interpretation, and which makes it possible that results will not alter preconceptions. In the correspondence for the same issue in which Huxley's reply to *Nature's* earlier editorial on his address appears (29 September, page 366), C. J. Robbins subscribes to the widely-held view that science sets out to falsify theories through experimentation. As the pages of *Nature* show, this is rarely true; the commonest motivation in research might well be exactly the converse.

The concept of IQ itself is a simplistic parameter which assumes that cognitive, perceptual, deductive and mathematical abilities go hand in hand, whereas our selection of individuals (for employment, etc.) shows this cannot be the case. We accept that brilliant mathematicians may be absent-minded, that musicians may be hopeless fine artists, scientists poor communicators. Why then do we hear so little about what we might call intelligence type? Until we have evolved realistic codes of criteria for assessment that reconcile mental measurements with the realities of life I believe we should postpone spurious research into racially-determined IQ, and recognise it as being ill-founded and premature.

In my view this is the most objective manner in which one could admit the limitations of contemporary science, and the irrelevance of research findings to those determined to subscribe to their own beliefs; these two are topics that are ripe for research.

Yours faithfully,

BRIAN J. FORD

Science Unit, Cardiff, Wales

Soviet genetics

SIR,—As far as I know, gerontologists have been wary, over the years, of passing opinions on human genetics and on human genetics programmes. I was therefore very interested to read the review by Dr Zhores Medvedev, a noted gerontologist, on the development of human genetics in the USSR since Lysenko ('Soviet genetics: new controversy', 28 July, page 285).

The review bears a title which is frightening for anyone knowing the history of genetics in that country. A new controversy is hardly needed at this stage. The international congress of genetics is due this year in Moscow

and even calling attention to controversy might cause unpredictable side effects. Fortunately, renewed anxiety for the fate of human genetics in the USSR seems premature at this stage; the review gives a brief account of the re-emergence of the discipline, and for the rest is an all-out attack on the retiring director of the Institute of General Genetics of the Academy of Sciences, Dr N. Dubinin.

Of those who reinstated human genetics in the USSR Medvedev writes: "all of them were void of real practical knowledge of human or medical genetics", and one is left to wonder how they happened to have the courage to do it. Furthermore, if those who only had knowledge of drosophila genetics, of radiation, rodents, cytology and theoretical human genetics can be criticised for their contribution what can a gerontologist say about human genetics; in what position is he to pass value judgements?

Dr Medvedev, although he does not say so, must have participated in the discussions, since he says they were peaceful, albeit not very productive. Again one wonders how the new Institute of Medical Genetics was established as an outcome of such a lack of productivity.

One cannot help noticing the amount of negative emphasis put by Dr Medvedev on an action which tended to reconstruct human genetics. It is my opinion that, since these negative emphases diminish the whole operation, he should produce the evidence on which they are based. What, for example, is the evidence that D. K. Beliaev had been appointed in 1976 president of the 1978 Congress? This is puzzling, because Beliaev is general secretary, and Tsitsin (wheat hybrids at Lysenko's time) is president.

The part of the review which is devoted to Dubinin is less important. Dubinin, as others, went through the bitter years of the Soviet geneticists and survived. At present, he seems to have developed his own personal views on the inheritance of human abilities; these views are questionable but since they have been publicly castigated, they are not official views which might endanger those not sharing them. It seems arbitrary to associate them with a controversy which might harm human genetics; he is entitled to his opinions, no less than Medvedev to his own and I to mine. So far as some of Dubinin's work on human genetics is concerned, and so far as I can read, he has recently used erroneous techniques in the study of multivariation in quantitative traits in man. However, it might well be that, as director, he signs work from his institute which might be beyond the capacity of his technical

judgment (for example *Dokl. Akad. Nauk.* 230, 4: 957-960, 1976).

I believe that the most contradictory aspect of Medvedev's review is that Dubinin is turning against the programme he helped to start a short time ago. I believe that the accusation Medvedev makes, that "Dubinin is working hard to suppress by all possible means the development of genuine research in the field of human genetics in the USSR", and which amounts to public condemnation, is so important, that Medvedev is bound to produce evidence for it. It would be sad for those who have helped in the reconstruction, even for those who have given a minimal contribution, if attempts to create new conflicts hinder development. Human genetics is a hot science to handle: it seems useless to make it even hotter. After all, state and interstate budgets for human genetics can be cut easily, both east and west of Greenwich.

Yours faithfully,

ITALO BARRAI

Universita di Ferrara,
Italy

The Messinian salinity crisis

SIR,—Lines in reply to an anonymous critic (20 October, page 646).

When composing lyric verse

Be it critical or worse

It is wise to be quite certain of one's ground,

And not to call absurd

Quite a harmless little word

Lest its meaning be not simple but profound.

Crises evaporitic

Irritate our nameless critic

Rightly so, had we but meant what he implied,

But salinity increased

Until crisis point was reached

Whereupon the fauna disappeared or died.

Oh, 'tis pity I declare

That whatever we prepare

And however clear the message that we send,

There are always colleagues who

Having little else to do

Criticise us when they do not comprehend.

N.B.—The term 'Salinity Crisis' was, I believe, coined by Ruggieri (Systematics Association Publication No. 7 (eds Adams, C. G. and Ager, D. V.), 283-290 (London, 1967)) and referred to the apparent extinction of the marine faunas of the western Mediterranean in Messinian times.

Your faithfully,

C. G. ADAMS

British Museum (Natural History),
London

news and views

T cell hybrids: shortcut or dead end?

from F. Melchers

THREE years ago everybody believed that normal lymphocytes could not be fused with malignant lymphocytes in culture. Then Köhler and Milstein (*Nature* **256**, 495; 1975) successfully fused an immunoglobulin-secreting myeloma with normal B lymphocytes which secreted antibodies specific for sheep red cells. This encouraged many laboratories to produce fused B cell lines secreting antibodies with a variety of required specificities (see *News and Views* **266**, 495; 1977). These 'hybridomas' (or Köhler lines perhaps?) represent a breakthrough in immunological research. Effector functions of clones of particular lymphocytes, in this case specific antibody production by B cells, can be made immortal. Consequently the clones can be grown up in large scale preparations that will allow structural and functional analyses of the clonal products.

It is difficult to say how this success came about after all the failures. Improvements in tissue culture techniques and selection of suitable malignant cell lines for fusion certainly helped. Köhler and Milstein recognised (*Somatic Cell Genet.* **3**, 303; 1977) that malignant and normal lymphocytes used in the fusion had to be at a comparable stage of differentiation. As a simple rule B cells, but not T cells, could fuse with B myelomas.

Once the ice had been broken everybody was eager to try similarly to fuse normal T lymphocytes and their malignant counterparts. This has now been achieved in several laboratories (Goldby *et al.* *Nature* **267**, 707; 1977; Hämmerling *Eur. J. Immun.* **7**, 743; 1977; Köhler *et al.* *Eur. J. Immun.* **7**, 758; 1977). For these fusions a suitable malignant cell line with T lymphocytic markers had to be found. Robert Hyman from the Salk Institute in La Jolla provided it in the T lymphoma BW5147. Fusion between normal T cells and this cell line was demonstrated by the codominant expression of genetic markers which

differ in the two cells, such as histocompatibility antigens, the T-cell specific Thy-1 marker and isomeric forms of the enzyme glucose-6-phosphate isomerase.

The ultimate aim of these fusions, however, is to preserve the effector functions of the parent T lymphocytes and thereby create cell lines with which the different effector functions of T lymphocytes could be elucidated. This, however, has proved less easy than had been anticipated. None of the T-cell hybridomas has so far revealed any effector function expected for normal activated T cells. There might be several reasons for this failure: the genome of the tumour cell might suppress the expression of the effector functions of the normal T cells; the chromosomes of the normal cells carrying genes for these effector functions might be lost after fusion and during growth of the T hybridomas. It is also possible that we are unable at present to fuse normal, activated T lymphocytes expressing a given effector function with T lymphoma cells or to assay for T cell effector functions *in vitro* when these functions are provided by individual clones of T cell hybridomas.

Three major immunological functions are attributed to T lymphocytes: help and suppression of B cell growth and maturation and killing of target cells. All three functions are thought to be initiated through clonal selection of specific T cells by antigen, resulting in the growth and differentiation of clones of antigen-specific T cells.

How, then, are these functions to be detected '*in vitro*'? First and most important, no single cellular assay exists for any T cell function as it does for immunoglobulin-secreting B cells in the form of Jerne's haemolytic plaque assay. Killer cells are most often monitored by ⁵¹Cr-release by labelled target cells. This method has recently been improved so that between 50 and 250 specific killer cells can now be detected in one culture, allowing *in vitro* analysis of the frequencies of target cell-specific killer T cell clones

(Teh *et al.* *J. Immun.* **118**, 1057; 1977). The general cytolytic activity, independent of the antigen-binding step, of killer to target cell can also be measured with a ⁵¹Cr-release assay. Killer and target cells in this non-specific assay are brought into close contact by agglutinins which act as a glue (Forman & Möller *J. exp. Med.* **138**, 672; 1973; Bevan *et al.* *Eur. J. Immun.* **6**, 150; 1976). The antigen-nonspecific cytolysis which occurs when the killer and target cells are in close contact, is precisely what seems to make successful fusions between activated killer T cells and a malignant T cell line impossible, since fusion necessarily involves close contact of the two cells to be fused. Köhler and his collaborators (*Eur. J. Immun. op. cit.*) have now shown that the close contact of activated T killer cells with the BW5147 T lymphoma line during the fusion reaction kills the T lymphoma cells and thereby prevents probably all killer cells, regardless of their target specificity, from becoming immortal. There is little hope of this nonspecific killing mechanism ever being inactivated during the fusion reaction, allowing the establishment of T cell hybridomas with killer activity.

In vitro assays for T cell help or suppression of B cells seem even less sensitive than killer assays. Furthermore, there still exists considerable controversy over the mechanism of T cell dependent B cell triggering (at its height in *Transplant. Rev.* **23**, 1975). Depending on which immunologist one talks to, quite different structures and actions are expected from the products of a T cell hybridoma. Many immunologists believe that T cells produce antigen-specific factors, which for a long time were thought to be immunoglobulin. This now seems less likely. Some people think that such factors carry Ia antigen, but others cannot find these Ia antigens on their factor preparations. Many also believe that these antigen-specific T cell factors in some way induce B cell growth and maturation to immunoglobulin secretion and are therefore mitogenic for B cells.

This all argues for soluble products effecting T cell help and suppression, but not everyone is yet convinced that cell-cell contact can be ruled out as the basis of the physiological action of T-B collaboration. Conceptual confusion prevails in a situation in which convincing experiments are still somewhat scarce.

The chances of finding a T-cell hybridoma which produces helper factor for a given antigen are indeed very low—approximately 1 in 10^4 for the antigen sheep red cells (Lefkovits & Waldmann *Immunology* **32**, 915; 1977). No wonder then that the few T hybridomas which have been tested so far for such antigen-specific helper functions do not produce them (Köhler *et al. op. cit.*). If these factors are mitogenic for B cells it might be predicted that, at higher concentrations, they could polyclonally activate all B cells which are receptive to the mitogenic principle of the factor regardless of the specificity of these B cells for the hapten. Assays for B cell mitogeni-

city have become very sensitive (Andersson *et al. Cell* **10**, 27; 1977) and such assay systems might be useful to detect growth-inducing properties of factors produced by T cell hybridomas, or even by T lymphomas.

However, one may still hope that T cell hybridomas will open up a new way to an understanding of normal T cell effector functions of help and suppression and their molecular mechanisms of action. Binding of antigen to T cell hybridomas or to molecules released by them seems one simple first step. Selection of T lymphoma lines which display nonspecific helper or suppressor activities with normal B cells, may also help—keeping in mind the 'rule' that cells at comparable stages of differentiation might fuse more easily with each other. Beyond that immunologists will have to replace ideology by scientific knowledge, obtained through better assays, so that they can detect what may be present in, on, or released by, T cell hybridomas. □

Maeyer-Guignard *et al. Proc. natn. Acad. Sci. U.S.A.* **69**, 1203; 1972). The assay can be made quantitative (Kronenberg & Friedman, *J. gen. Virol.* **27**, 225; 1975; Greene *et al.*, this issue of *Nature*, page 81). Second, both mouse and human interferon mRNAs have been translated in cell-free systems, mouse interferon mRNA in the wheat germ system (Thang *et al. Proc. natn. Acad. Sci. U.S.A.* **72**, 3975; 1975) and human interferon mRNA in cell-free extracts from mouse ascites cells, rabbit reticulocytes and wheat germ (Pestka *et al. Proc. natn. Acad. Sci. U.S.A.* **72**, 3898; 1975; Reynolds *et al. Proc. natn. Acad. Sci. U.S.A.* **72**, 4881; 1975; Raj & Pitha *Proc. natn. Acad. Sci. U.S.A.* **74**, 1483; 1977). Third, human interferon mRNAs have also been translated after injection into *Xenopus* oocytes (Raj & Pitha, *op. cit.*; Cavalieri *et al. Proc. natn. Acad. Sci. U.S.A.* **74**, 3287; 1977; Sehgal *et al. Proc. natn. Acad. Sci. U.S.A.* **74**, 3409; 1977), and both the cell-free assay and the oocyte assay can also be made quantitative. The products of these translation systems have been characterised as interferon using species and antigenic specificities, and sometimes also by showing the product to have the expected molecular weight. Use of the *Xenopus* oocytes showed that injection of polyadenylated RNAs from induced fibroblast and lymphoblastoid cells yielded fibroblast and leukocyte interferons respectively, demonstrating that human cells can produce two different translatable mRNAs and probably contain two genes for interferon, coding for polypeptides differing in primary sequence.

What has been learnt about the control of interferon formation using these translation systems? It is well established that the interferon mRNA is found in the polyadenylated RNA fractions, although there has been a report of activity also being associated with non-polyadenylated material (Montagnier *et al. Biochem. biophys. Res. Commun.* **59**, 1031; 1974). No translatable RNA has been found in uninduced cells, and therefore induction is accompanied by a rapid increase in the amounts of translatable RNA. Thus the switch-on of interferon synthesis requires new transcription; the alternative explanation that translatable RNA is processed from a pre-existing RNA species is ruled out because of the well known inhibitory effect of actinomycin on interferon production. Two groups (Sehgal *et al. op. cit.*; Greene *et al. op. cit.*) have also shown that interferon mRNA is undetectable 6–8 h after induction with double-stranded RNA. This suggests that the switch-off of interferon production is due to cessation of mRNA

Translation of interferon messenger RNA *in vivo* and *in vitro*

from Derek Burke and John Morser

INTERFERONS are induced when a large variety of animal cells are treated with viruses or double-stranded polyribonucleotides. Cells which, before induction, were producing no detectable interferon, will produce up to 10^5 reference research units of interferon per 10^6 cells in the 24 hours after induction. Cells that have been induced are switched-off a few hours later. A second dose of interferon inducer does not lead to a second yield of interferon; the cells are described as hyporesponsive. Production of interferon can also be modulated in other ways; pretreatment of cells with small amounts of the homologous interferon before addition of an interferon inducer often increases the yield, a phenomenon termed 'priming'. Certain metabolic inhibitors added after induction with a double-stranded RNA can have a similar effect, a phenomenon called super-induction. Since purified interferon has a high specific activity (approaching 10^6 units per mg protein) and since the bioassay can easily detect 10 units of interferon, the production of very small amounts of interferon can be measured. Interferons are usually species-specific: interferon made in

mouse cells is inactive on human cells and *vice versa*. But even within the interferons produced by one species, there may be heterogeneity, for there are at least three different types of human interferon, which are distinguished by the type of cell in which they are formed: fibroblast interferon, leukocyte interferon and immune (type II) interferon produced when lymphocytes are treated with mitogens. Lymphoblastoid cells produce mainly the leukocyte type. These three interferons are antigenically distinct and have different physical and biological properties. The ease of detecting small amounts of interferon, the tight control of interferon production, and the existence of several different human interferons all make the interferon production system attractive for exploring the control of inducible proteins in eukaryotes. With the development of systems for translating interferon mRNA *in vitro* interesting answers about the control are beginning to emerge.

Three techniques have been developed for translating an interferon mRNA *in vitro*. The first involves application of RNA extracted from interferon-producing cells to heterologous cells with production of interferon characteristic of the cells from which the RNA was extracted (De

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synthesis as well as to its inactivation.

The levels of interferon mRNA have also been measured in normal and superinduced cells, in order to determine whether the increased interferon production is due to a corresponding increase in the amount of mRNA. Raj and Pitha (*op. cit.*) found that the levels of interferon mRNA at 6 h after induction were very much higher in superinduced cells than in normal cells when the wheat germ system was used, but that there was little difference when the *Xenopus* oocyte system was used. In contrast Sehgal *et al.* (*op. cit.*) found the levels of mRNA were higher in superinduced cells using the oocyte system but they could not obtain translation of the same mRNA preparations in cell-free systems. Although there are differences between these two reports, both groups agree that the increased level of interferon production in superinduction is not due to the synthesis of new interferon mRNA, but is due to a change in the fate of the mRNA. Pitha and

Raj (*op. cit.*) suggest that similar levels of interferon mRNA are produced in normally induced and superinduced cells, but that the majority of the mRNA is in an inactive form in normally induced cells. They further suggest that superinduction is due to a modification of this RNA, which can also take place in *Xenopus* oocytes, but not in the wheat germ translation system.

However, Sehgal *et al.* (*op. cit.*) suggest that in normal cells mRNA is being degraded or inactivated but that superinduction prevents this process, through the action of a repressor gene. Although there are clearly still some difficulties in correlating the *in vivo* and the *in vitro* results, the experiments have already led to models of the control of interferon synthesis which can be tested. In the longer term, more detailed analysis will require other approaches, for example the use of nucleic acid hybridisation techniques to measure the amount of interferon mRNA directly. □

At that time, a number of endemic African forms moved out to occupy the Eurasian land mass. Hominoids of the *Proconsul* type were apparently among these emigrants and they rapidly spread very widely.

Recent work in Hungary (Kretzoi *Nature* **257**, 578; 1975), Greece (de Bonis *et al. Cr. Séanc. hebdom. Acad. Sci. Paris Ser. D.* **281**, 379; 1975; **284**, 1393; 1977) Spain (Crusafont-Pairo & Golpe-Posse, *J. hum. Evol.* **2**, 17; 1973), Turkey (Tekkaya *Bull. Min. Res. Exp. Ankara* **83**, 148; 1974; Andrews & Tobien, *Nature* **268**, 699; 1977) and on the Potwar Plateau has greatly increased our knowledge of this hominoid radiation.

It is now apparent that at least three genera, *Sivapithecus*, *Ramapithecus* and *Gigantopithecus*, may be recognised in deposits reflecting the more open woodland habitats of that time. These three genera share a complex of characters which may reflect a dietary adaptation to the harder or coarser foods of such environments. These features include thick enamel on the teeth, cheek teeth which were large in relation to body size and a mandible which was heavier and thicker than that of their more ape-like contemporaries.

From our point of view, the most interesting of these woodland-adapted genera is *Ramapithecus*. This genus is considered by many to be the earliest known member of the Hominidae, the family to which man and his phylogenetic ancestors belong. Indeed, as Pilbeam points out, unworn cheek teeth of this middle Miocene form very closely resemble those of later hominids such as *Australopithecus* and *Homo*. The earliest known member of this genus, and the family Hominidae, was recovered at Fort Ternan, Kenya just below a level dated with potassium/argon to 14 Myr BP. It has recently been suggested that an even earlier member of the genus may occur at Pasalar, Turkey (Andrews & Tobien *op. cit.*). However, the date of these Turkish deposits is based on faunal remains and it must be pointed out that not all elements of the Pasalar fauna confirm the suggested date of 15 Myr. For example, the bovid taxon *Prostrepsiceros rotundicornis* may occur at Pasalar but it does not appear elsewhere in Europe until about 10 Myr ago. If this species does indeed occur at Pasalar then a date for these deposits of 15 Myr must be treated with extreme caution.

However, the suggestion by Andrews and Tobien of a non-African origin for the hominids is an intriguing one. The genus *Ramapithecus* is known only from a single site in the whole of Africa and it is there found in association with a fauna that has many im-

Hominoid habitat shifts in the Miocene

from G. E. Kennedy

THE middle Miocene was a time of widespread environmental shifts which resulted in profound changes in many faunal groups. The tropical and subtropical forests of the earlier Cenozoic had provided large and virtually unbroken tracts of lush, protective habitat for many animals. But by the middle Miocene, around 9–14 Myr ago, a general cooling trend, coupled with tectonic and mountain building activity, had fragmented these forests into smaller, discontinuous habitat areas. With the decline of this habitat space, many animals moved from their long-occupied forest niches into the newly expanding, more open woodland areas. Among the Miocene faunal groups which made a shift from forest to open woodland were some groups of the Hominoidea—that primate super-family which contains apes and man, both living and fossil. In a recent issue of *Nature* (**270**, 684, 689; 1977) Pilbeam *et al.* report on some middle Miocene hominoids from the Potwar Plateau, in the Punjab Province of Pakistan which had made such a shift.

Hominoids of Miocene age have been known to palaeontologists since 1856. Their diversity, both morphological and geographical, led to a pro-

liferation of taxa which made the group unwieldy, if not incomprehensible, even to specialists. A revision of their taxonomy in 1965 (Simons & Pilbeam, *Folia primat.* **3**, 81) was a welcome move in increasing understanding of the interrelationships of this group, which may contain the ancestors of living gorillas, chimps and man.

However, Pilbeam's work and the work of other primate palaeontologists now indicates that the elegant simplicity of that taxonomic scheme may have underestimated the vigour of the adaptive radiation of the middle Miocene hominoids. Since that revision several new genera have been proposed and more recently it has been suggested that two genera, previously sunk to sub-generic level, should now be reinstated as full genera. One of these, *Proconsul*, was an African early Miocene forest-living form, apparently at or near the ancestry of most later hominoids. Pilbeam now suggests that the other genus, *Sivapithecus*, was among the hominoids which made a habitat shift during the middle Miocene. A similar suggestion has recently been made by Andrews in a paper given at the VI International Primatological Congress, at Cambridge, 1976.

The opportunity to make this shift was enhanced when the long geographical isolation of the African continent ended about 16–17 Myr ago.

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migrant, Eurasian elements. The geographical origins of the Hominidae should be a fascinating area of research in the coming years.

The environment in which the early hominids lived is also an intriguing area of study. Many theoretical discussions of hominid origins have been based on the development of an adaptive complex which emerged in response to pressures from savannah or grassland environments. However, as considerable recent research has indicated, grassland environments were not occupied by the earliest hominids and were, in fact, only entered by this group relatively recently. Therefore, occupation of the savannahs cannot have been the 'initial kick' which led to the emergence of the family Hominidae. Savannah adaptations may well have been important in the course of later hominid evolution, particularly in the Plio-Pleistocene radiation, but they cannot have been an initiating factor. Moreover, it is of interest and of uncertain significance that not all ramapithecines have been found in woodland deposits. In two areas, at Rudabanya, Hungary (Kretzoi *Nature op. cit.*), and, at Keiyuan, south China (Chow *J. paleont. Soc. Ind.* **3**, 123; 1958) this form has been found in lignite deposits indicative of very moist, heavily vegetated conditions.

Clearly, the adaptive radiation of the middle Miocene hominoids was very complex. The human ancestors, rather than showing a unique and singular pattern of evolution were, in fact, part of a much broader process.

'Cardiac' RNA

from Jonathan Slack

It has long been realised by developmental biologists that the formation of ordered patterns of cell types during embryonic development requires the existence of interactions between different parts of the early embryo. The chemical nature of the putative signals has been a tantalising mystery ever since the abortive 'gold rush' to isolate Spemann's organiser during the 1930s. To the biochemically minded, informational macromolecules such as RNA present themselves as obvious candidates, while to the adherents of 'gradient' models they seem to be a red herring, and the signals are generally thought to be small molecules or ions which unleash complex responses

from the target cells.

Two recent papers from A. K. Deshpande and M. A. Q. Siddiqui (*Dev Biol.* **58**, 230; 1977; *J. biol. Chem.* **252**, 6521; 1977) make up an interesting contribution to this debate. They have isolated a specific fraction of RNA which causes a specific type of cell differentiation in explants from early chick embryos. At the stage concerned (Hamburger and Hamilton's stage 4), a chick embryo consists of two layers of cells: the epiblast above and the hypoblast below. Joining these layers down the centre is a mass of cells called the primitive streak through which cells are migrating to form a mesodermal layer in between the other two. At the front end of the primitive streak is a condensation of cells called Hensen's node, which is supposed to be the 'organising centre' for the formation of the general body plan.

Deshpande and Siddiqui have isolated their RNA species from the hearts of quite advanced (16 day) embryos. It is about 7S (200 nucleotides) in size and contains a sequence of poly(A). When the 'post nodal piece', roughly the posterior third of the early embryo, is treated with this RNA *in vitro*, it differentiates into cardiac muscle tissue.

What distinguishes this result from claims of this type in the past is the careful characterisation of the response and the large number of controls. The positive cases show beating movements and contain myofibrils (electron microscopy), actin and myosin (gels), glycogen granules (histochemistry) and acetylcholinesterase (assay) in quantities appropriate to cardiac muscle cells. The posterior explants do not form cardiac tissue or anything much else when cultivated on their own, nor when treated with RNA from a variety of other sources, or with synthetic polynucleotides. However, it is not yet proved that the 7S RNA enters the cells in an intact form.

The intriguing thing about this result is that it makes little sense in terms of early development. The fate of the post nodal piece in normal development is to form the posterior axial structures—notochord and somites. When cultured on its own *in vitro* it does not differentiate at all, and when cultured in the presence of Hensen's node it will differentiate into axial structures (Butros *J. exp. Zool.* **149**, 1; 1962). It seems probable that this region is competent to form heart since the determination of the overall body plan is still going on at this early stage and classical work showed that quite an extensive lateral area would differentiate into heart when cultured *in ovo* on the chorioallantoic membrane (Rawles *J. exp. Zool.* **72**, 271; 1936). But according to our present ideas about pattern formation the position of

the heart rudiment should be specified by two distinct signals. This is because the paired rudiments are situated in a two-dimensional sheet of cells and located symmetrically on either side of the axis. There is no particular reason why any factor involved in these early signals should be present in 16-d heart at all. Significantly enough, the authors show that brain, liver and kidney RNA are all inactive, both in stimulating heart differentiation and in stimulating differentiation of their own cell types.

The 7S RNA itself does not seem to be a mRNA even though it contains a poly(A) sequence, since it cannot be translated *in vitro* and actually inhibits the translation of purified messengers in cell free systems. It will be most interesting to see whether this species exists in the early as well as the late embryo, for if it has a role in normal development it should be possible to find it there. □

First interstellar NO bond

from Graham Richards

THE list of interstellar molecules grows longer and longer and many large polyatomic systems have been positively identified with still more postulated. Despite this, NO bonds have been conspicuously absent from the lists and NO itself has been the subject of many unsuccessful searches. Now with the detection of HNO by Ulrich, Hollis and Snyder (*Astrophys. J.* **217**, L105; 1977) this anomaly in the chemistry of interstellar molecules may be removed.

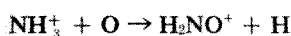
The observations were made in March, 1977 with the 36-foot radiotelescope of the National Radio Astronomy Observatory. The single line ascribed to HNO was detected in emission from the sources known to astronomers as SgrB2 and NGC2024. In general it is difficult to identify a new molecular species positively on the basis of a single spectral line but in this case very accurate laboratory frequencies are available (Saito & Takagi *J. molec. Spectrosc.* **47**, 99; 1973). Further transitions of HNO may require observations at millimetre wavelengths.

Comparison with the very similar molecule HCO suggests an abundance ratio of carbon to nitrogen of about 1.4 in the same interstellar clouds, which conforms with cosmic abundance estimates. The chemical simi-

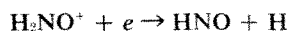
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Graham Richards is a lecturer in the Physical Chemistry Laboratory of Oxford University.

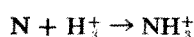
larities between HCO and HNO in all probability do not extend as far as the formation mechanisms. The formation of HCO is likely to depend on the availability of ionised carbon in moderate-density clouds. Ionised nitrogen may not be so readily available, so that the best suggestions for HNO formation come from Dalgarno



followed by



the initial NH_3^+ being derivable either from neutral nitrogen



or from the ion, N^+ , and unionised hydrogen molecules.

The letter in the *Astrophysical Journal* also reports a new unidentified line at a frequency of 81,505 MHz which may be a weak maser and observations of previously detected transitions of ketene (H_2CCO), dimethyl ether (CH_3OCH_3) and cyanoacetylene (HCCCN). A further unidentified line previously reported by Turner (*Astrophys. J. Lett.* **213**, L75; 1977) was also observed and ascribed to thioformaldehyde (H_2CS) or cyanoacetylene ($\text{HC}_2^{13}\text{CN}$).

The ambiguous identification, the unidentified line and above all the detection of an interstellar NO bond will all provide spurs for theoreticians to perform accurate calculations, spectroscopists to measure frequencies as well as encouragement for radio-astronomers to confirm and extend this study, perhaps once more searching for nitric oxide in interstellar regions.

Hydrothermal waters and heat flow

from Peter J. Smith

THE heat flowing outwards through the oceanic crust reaches the Earth's surface in three ways—by conduction, convection and radiation from the mantle and core, by conduction from radioactive sources in the crust itself, and by direct transport in the hot mantle material which rises at active oceanic ridges to form new lithosphere. Of the three, the most important is the last; indeed, more than 50% of the Earth's heat loss (that is, from oceanic and continental regions combined) takes place at oceanic ridges alone. With the possible exception of the few

Speed of light and relativity

from P. E. Hodgson

EINSTEIN'S special theory of relativity is based on two postulates—first that the laws of physics that hold in one coordinate frame also hold in any other coordinate frame moving with uniform velocity with respect to it, and second that the speed of light is independent of the velocity of the source. From these two postulates the theory of relativity can be derived.

The second of these two postulates can be tested experimentally and this has already been done in several ways. Among the most accurate is the study of gamma rays emitted from the decay of relativistic uncharged pions produced in very energetic nucleon-nucleon collisions. In one of these measurements the pions had an energy of 6 GeV and thus a velocity very nearly that of light, so that if the velocity of the source is added to that of the light, the gamma rays from their decay would have nearly twice the velocity of light, whereas if the velocities do not add the velocity is just c . There is thus nearly a factor of two between the velocities expected on the two possibilities, and this can be measured accurately by a time-of-flight method. If the result is expressed by saying that the measured velocity is

$$c' = c + kv$$

where k is a constant and v the velocity of the source, then the two possibilities correspond to $k = 0$ and $k = 1$. The measurements showed that k is smaller than 10^{-4} , which confirms the second postulate to high accuracy.

It is, however, always interesting to see if such an important result can be checked to even higher accuracy, and this has recently been done by Kenneth Brecher of the Center for Space Research at Massachusetts Institute of Technology (*Phys. Rev. Lett.* **39**, 1051; 1977). He made use of the regularly pulsing X-ray sources in binary star systems. Since in a binary star one component is orbiting round the other, if the system is observed in the plane of the orbit, for part of the period one star appears to be moving towards us and the other away from us, and the opposite happens half a

period later. If the velocity of the source is added to that of the light then we would not see two stars rotating around each other but instead would see complicated effects depending on the distance of the stars. In particular we would not see them continuously: they would appear and disappear as the various beams from the stars in different positions overtake one another as a result of their different velocities.

This method of confirming that the velocity of light is independent of that of the source was suggested long ago, but for light in the visible region it suffers from the objection that the radiation from a distant star is scattered as it goes through the intervening interstellar matter, and is then replaced by reradiated fields produced by the dipoles of the medium. This wave is then propagated with the phase velocity of the medium. Calculations show that for light in the visible region the characteristic length for the extinction of the original radiation is about two light years, which is less than the distance of the nearest star.

This objection does not apply to the 70 keV X rays emitted from some binary star systems, as they are much more penetrating and the interstellar extinction is negligible. Such stars thus provide a very sensitive way of testing the second postulate.

Brecher applied this method to three binary X-ray sources, and determined their distances, orbital periods, and Doppler velocities. A small but finite value of the constant k would affect the eclipse times and the apparent eccentricity of the orbit, and so measurements of these quantities enable upper limits to be set to k . A careful analysis of the results of the measurements showed that for one of the stars k is less than 2×10^{-9} , and for the other that it is less than 4×10^{-10} . These limits are much less than those set by the pion method. The second postulate is thus established to much greater accuracy than ever before.

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local continental areas of high heat flow capable of providing geothermal power, oceanic ridges are therefore the most interesting of all the Earth's thermal features. They are also the most important in the sense that study of their thermal characteristics is capable in principle of throwing light on

some of the details of the plate tectonic processes which brought the heat to the surface in the first place.

Unfortunately, however, oceanic ridges are precisely those regions where heat flow measurement is most difficult and the results most uncertain. Conventional heat flow probes require for

their operation at least several metres of sediment, but the new lithosphere in ridge zones has had little time to develop a sediment cover. Measurement is therefore limited to the small isolated basins where sediment has begun to form, which are by definition not typical of the whole region. Moreover, the rugged ridge topography, which allows such sediment pools to grow and which is itself the product of variable thermal expansion, distorts the flow of heat and thus introduces geographical variations that are difficult to correct for. Small wonder, therefore, that heat flow data from ridges, though generally high as predicted by plate tectonics, are both highly scattered and subject to systematic errors which generally do not even permit calculation of a true mean value.

Nor is that all. Some of the systematic deviations (for example, the conspicuously low heat flow often found on ridge flanks) are so great that it is impossible to account for them either by topographic distortion or as the result of the accumulation of cold sediment. This has led several workers to suggest that an important influence on the distribution and variation of heat flow in the vicinity of oceanic ridges is hydrothermal circulation in the igneous upper crust—an idea put forward many years ago by Elder (in *Terrestrial Heat Flow*, edit. by Lee, W. H. K., American Geophysical Union, 1965) but promoted particularly vigorously in recent years by Lister (*Geophys. J.* **26**, 515; 1972 and subsequent articles). Lister's point is that heat advected from the crust by hydrothermal circulation which has open access to the ocean water will not be recorded by measurements limited to sediment pools. Average heat flow values obtained from ridge zones will therefore almost always be too low to be consistent with otherwise reasonable models of lithospheric accretion which ignore hydrothermics. Only when there is a complete sediment cover forming a continuous impermeable barrier between the igneous crust and the open ocean will the measured average heat flow begin to approach the true value of heat lost by the oceanic lithosphere.

The existence of hydrothermal circulation is now widely accepted, having been demonstrated to be the most likely explanation for the heat flow distributions revealed by several studies since 1972. A particularly striking confirmation of Lister's basic ideas, however, has now been reported by Davis and Lister (*J. geophys. Res.* **82**, 4845; 1977) who have obtained 104 heat flow results from a 110 km square area centred on the point where the Juan de

Fuca ridge meets the transform (Sovanco fracture zone) that offsets it from the Explorer ridge to the north. In the southern section of this area lies the northern end of the Juan de Fuca ridge with its young lithosphere (0–1.8 Myr old), whereas to the north of the fracture zone lies the older crust (3.3–3.6 Myr) which has spread from the Explorer ridge. In other words, the test area includes two quite distinct zones between which the effects of hydrothermal circulation, if any, may be compared.

Throughout the area as a whole the heat flow observations, made at roughly 10 km intervals, were highly variable, ranging from 1.4 to 15.9 $\mu\text{cal cm}^{-2} \text{s}^{-1}$ and having no obvious correlation with either topography or sediment cover. Moreover, this variability and lack of correlation were equally evident in eight measurements made specially at 2 km intervals. From this evidence alone it seems necessary to invoke hydrothermal circulation throughout the test area.

But the consequences of the circulation differ significantly between the two subzones. The overall picture is mirrored south of the fracture zone where the sediment distribution over the Juan de Fuca ridge and ridge flanks is intermittent and the sediment, where it exists at all, is thin. The heat flow variability is far too great to be explained solely in terms of topographic distortion or sedimentary accumulation. Where there is no sediment cover there must be open circulation between the hydrothermal waters in the igneous lithosphere and the ocean waters above, leading to an underestimate of the true heat flow by the actual measurements made in sedimentary areas. This is confirmed by plotting the measured mean heat flow against the reciprocal of the square root of the age of the lithosphere. The point thus produced lies way below the line derived from other Pacific heat flow provinces where there is no hydrothermal circulation.

North of the Sovanco fracture zone, on the other hand, the basement of the east flank of the Explorer ridge is completely covered with thick flat-lying sediment. The heat flow here is still variable, but rather less so than in the south of the test area; and there is now a distinct inverse correlation between heat flow and the corresponding sediment thickness. In principle, both the variability and the correlation could be explained either by topographic distortion (by which heat tends to converge towards areas where, because of topographic variations in the basement, the sediment is thinner and hence its insulating effect smaller) or by the cooling influence of sedimentary deposition. Indeed, the presence of the heat flow–sediment thickness correlation in par-

ticular points strongly towards just such explanations. Calculations show, however, that even in this zone these effects are insufficient.

So it is necessary to invoke hydrothermal circulation even though in this case there is a complete sediment cover. But this is not to say that the sediment cover has no effect. On the contrary, it prevents the hydrothermal waters in the igneous lithosphere venting to the ocean and thus eliminates the false measurement of heat flow inevitable in the case of the Juan de Fuca ridge zone. The measured average heat flow is thus the true heat flow; and this is confirmed by the fact that the former plots precisely on the Pacific heat flow–lithospheric age curve.

In summary, then, what Davis and Lister have shown, above all, is that artificially low values of measured heat flow are entirely consistent with the effects of hydrothermal circulation but that such circulation does not give rise to artificially low values of measured heat flow in all circumstances. Although hydrothermal circulation will always produce scatter of heat flow, a false average value will result only when the hydrothermal waters can vent to the ocean. Incidentally, such venting will, not surprisingly, also lead to a reduction in the average temperature of the hydrothermal waters themselves. Thus Davis and Lister estimate that temperatures at the top of the igneous crust range from 300 °C to 100 °C below the few well-sedimented sections of the Juan de Fuca ridge but from 200 °C to below 25 °C where there is no sediment cover. □



A hundred years ago

THE vessel was at this time in the neighbourhood of Terra del Fuego, about 140 miles from Magellan's Straits, when early in the morning it narrowly escaped collision with an island where no trace of land appeared on the charts. The vessel hove-to until daylight, when the captain proceeded with a boat's crew to the new island, which had gradually diminished in size since the first observation. Around the conical rocky mass the water was hissing, and although no smoke appeared, it was found to be too highly heated to permit of landing. The sinking continued slowly, until at eight o'clock the island was completely submerged, and an hour later the vessel passed over the spot where it had disappeared.

From *Nature* 17, 3 January, 194; 1878.

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review article

Prediction of protein structure from amino acid sequence

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In principle, it is possible to predict theoretically the three-dimensional structure of a protein from its amino acid sequence. Recently substantial progress towards this goal has been made by the use of simple models to represent protein conformation and interatomic interactions, together with the knowledge gained from analyses of known protein structures.

THE distinguishing feature of biological macromolecules, especially proteins, is their ability to adopt a unique three-dimensional structure (Fig. 1). It is this precise structure which is essential for recognition between different molecules in an organism and determines how they function. Thus knowledge of tertiary structure is vital for understanding biological function. Experimental evidence shows that all the information necessary for a protein to acquire its complex structure is stored in its linear amino acid sequence¹. Therefore theoretically one should be able to predict the conformation of a protein from a knowledge of sequence alone. Once a protein has been isolated, its sequence can almost always be determined. In contrast, structure determination is difficult and many proteins defy all attempts to obtain suitable crystals. Thus a successful prediction of three-dimensional structure, which implies a basic understanding of how structures are formed, would be an important step forward in molecular biology.

This review will examine the progress made in the prediction of protein structure from amino acid sequence over the last five years (for other reviews see refs 2–4). The major problem of prediction is that even a small protein, 50 amino acids, could adopt about 10^{50} conformations. How does one find the native structure amongst the myriad of alternatives? Recent progress towards a solution of this problem has been made by use of simple models to represent both the conformation and interatomic interactions of the protein, together with the knowledge gained from the increasing number of protein structures known.

Thermodynamics and kinetics of refolding

Many experiments^{1,5} have shown that most proteins can spontaneously refold in a biological environment, from a denatured state to their native conformation. Generally this is true for proteins with and without disulphide bridges and for both monomeric and multi-subunit proteins. These experiments led to the thermodynamic hypothesis¹, which states that the polypeptide chain folds so as to attain the conformation of lowest free energy. This hypothesis was modified in 1968 by Levinthal⁶, who also considered the kinetics of folding. *In vivo* proteins require between 10^{-1} and 10^3 seconds to fold up, and, since molecular rearrangements occur in about 10^{-13} s, a protein cannot possibly search all conformations ($> 10^{50}$) (ref. 7). Thus folding must be directed along pathways and the native structure will be at the minimum free energy of the kinetically accessible conformations. This local minimum need not necessarily correspond to the global minimum of free energy. To reduce the number of conformations searched it is thought that portions of the chain serve as

nucleation sites, around which the remainder of the protein folds. The α -helix and β -strand are possible sites. One suggestion for the pathway, called N-terminal folding, is that the polypeptide chain folds as it is being synthesised from its N-terminus⁸. Definitive experimental evidence on folding pathways has proved difficult to obtain. Studies on pancreatic trypsin inhibitor⁹ and hen egg white lysozyme¹⁰ suggest that at most there are only a limited number of folding pathways to the tertiary structure.

Energy calculations for atom–atom interactions

Theoretical predictions of structure have been influenced by both the thermodynamic and kinetic arguments. Initially the thermodynamic properties of polypeptide chains were considered. Liguori¹¹, Ramachandran¹² and their coworkers showed that the peptide unit can adopt only certain allowed conformations (Fig. 1d). By considering non-bonded interactions, they constructed ϕ – ψ plots which have subsequently been improved by semi-empirical energy calculations¹³ to predict preferred regions. There is good agreement between these preferred conformations and the observed distribution of ϕ , ψ angles. Recently energy calculations have been refined, either by choosing parameters to fit crystal structures¹⁴ or by performing complex molecular orbital calculations¹⁵.

It follows from the thermodynamic hypothesis, that by calculating the total free energy of a protein and finding the global minimum, it should be possible to predict the native structure. But at present this approach is not feasible. Not only is the computer time required excessive, but the energy surface is extremely complex with numerous minima. Consequently studies have been restricted to small sections of proteins and polypeptides¹⁶, and the refined calculations have only been valuable in the understanding of local conformation. The multi-minimum problem will probably be solved by finding the correct folding pathway. One step towards this goal would be the prediction of regular secondary structures from the sequence, since these structures may form the nucleation sites (see for example ref. 17).

Prediction of secondary structure

More than fifteen methods of predicting secondary structure from amino acid sequence have been proposed. (References are given to some of the more recent approaches: 18–27.) These predictions assume that local sequence determines local structure, which has been partially verified by the results. Usually only short-range interactions are considered, interactions between distant chain segments being ignored.

Broadly the methods can be divided into two categories. First, there are the statistical methods. In proteins of known sequence and structure, each residue is assigned to one class of secondary

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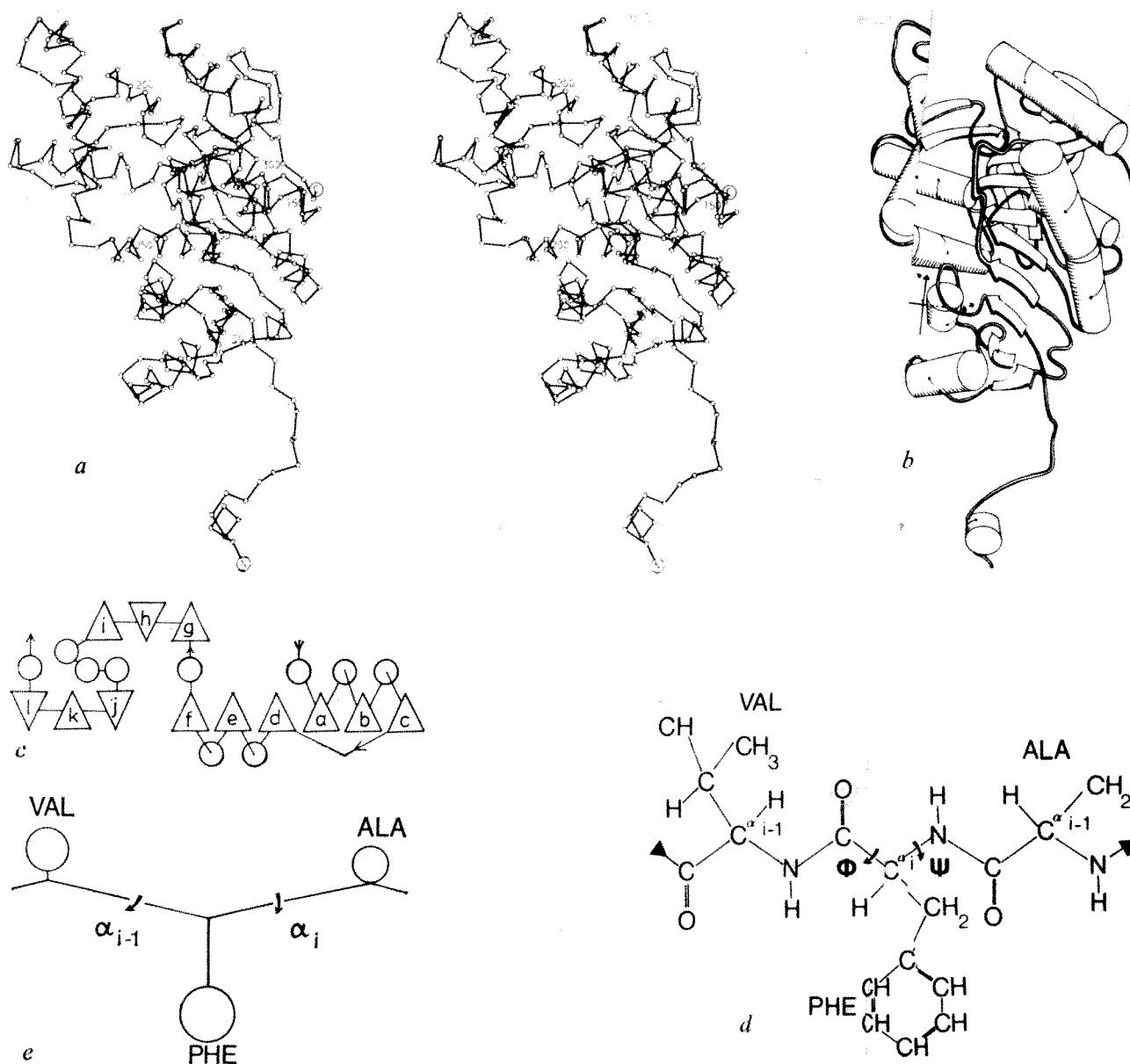


Fig. 1 Protein Structure. The figure shows how the complex three-dimensional structure of a protein—(lactate dehydrogenase (LDH))—can be simplified for use in analysis and prediction. *a*, A stereo diagram of the main chain (C² atoms) of LDH (to be viewed with stereo glasses). *b*, Schematic diagram of the main chain of LDH in the same orientation as Fig. 1*a*, showing the relative positions of the α-helices (cylinders) and β-strands (ribbons). *c*, A highly schematic diagram of LDH showing the relative positions of α-helices (○) and β-strands (Δ and ▽ according to whether viewed from the N- or the C-terminus of the strand). *d*, A section of polypeptide chain showing all the atoms and the dihedral angles φ and ψ. *e*, Simplification of (*d*) used by Levitt and Warshel^{52,55} to predict structure.

structure: α-helix, β-strand, coil, and sometimes β-turn. The observed distribution of amino acids amongst these classes is then used to derive parameters for the probability that a particular amino acid type will adopt a given class of secondary structure. The methods vary both in how the parameters are derived and how they are then used to predict structure. Some schemes sum the effects of individual residues^{18–20} while others also incorporate pair and/or triplet information^{21–24}. The method of Chou and Fasman¹⁹ has attracted most attention as it is simple to understand, can be applied without a computer, and has been relatively successful.

Second, there are a few methods^{25–27} which do not incorporate statistical data but are based on stereochemical criteria. Lim²⁷ has considered both the hydrophobicity and size of side chains to propose favourable patterns of residues that will form α-helices and β-strands. This apparently complex method, once understood, can be applied easily without a computer. It is appealing because it emphasises the importance of the positions of hydrophobic and hydrophilic residues and also incorporates, to some extent, long-range interactions.

Schulz²⁸ and Matthews²⁹ combined the results of several predictions to obtain joint prediction histograms for adenyl kinase and bacteriophage T4 lysozyme, respectively. The joint predictions were shown to be comparable with the best single prediction. This combined approach has been computerised by Argos *et al.*³⁰ who then applied it to 20 independent protein structures. An advantage of this completely computerised method is that it is impartial, whereas some of the individual schemes require human intervention to make the final assignments. The following generalisations about secondary structure prediction can be made.

No individual method is clearly superior. The success of a prediction for helices can be measured by a correlation coefficient C_α (ref. 29), where $C_\alpha = 1$, indicates a perfect prediction, while $C_\alpha = 0$, indicates a random prediction. C_β and C_T are similarly defined for predictions of β-strands and β-turns, respectively. The success of a given scheme varies considerably between proteins. Better predictions are generally obtained for small thermostable monomeric proteins than for the larger molecular weight structures. Argos *et al.*³⁰ find a range of values of $C_\alpha = 0.2$ to 0.86,

$C_\beta = 0.03$ to 0.73 and $C_T = 0.10$ to 0.84 . Thus the predictions were better than random for all structures. Although most of the α -helices and more than half of the β -strands and β -turns are located roughly, there are often errors in determining their termini. Presumably α -helices are predicted more accurately as local interactions are particularly important in their formation. Often the amino-terminal half of protein is predicted better than the carboxyl-terminal half, suggesting an amino-terminal nucleating core, in which short-range interactions dominate.

The success of predictive schemes has not greatly improved since 1974 although more protein structures are now available. This suggests that failure to achieve 100% accuracy is not the result of limited data, but that the formation of secondary structure is also determined by long-range interactions, which are not adequately considered.

Analysis of tertiary structure

The globular tertiary structure of a protein is stabilised by long-range interactions, which occur between non-local sections of sequence, for example the packing between two helices. With the increase in the number of protein structures known, many common structural features have been recognised, which were not previously predicted, and it has become apparent that proteins follow certain 'folding rules', which help determine tertiary structure. The simplification of the complex tertiary structure by the use of schematic diagrams (Fig. 1b, c) has helped in the understanding and comparison of protein structures. The results of analyses of tertiary structure, which are important for structure prediction, will be described.

Inspection of detailed interatomic interactions shows that two features are important determinants of protein structure. All proteins are closely packed, with packing densities comparable to those of small organic crystals³¹. This requirement for close-packing restricts the observed packing angles of α -helices and β -sheets to a few categories³². Second, hydrophobic groups tend

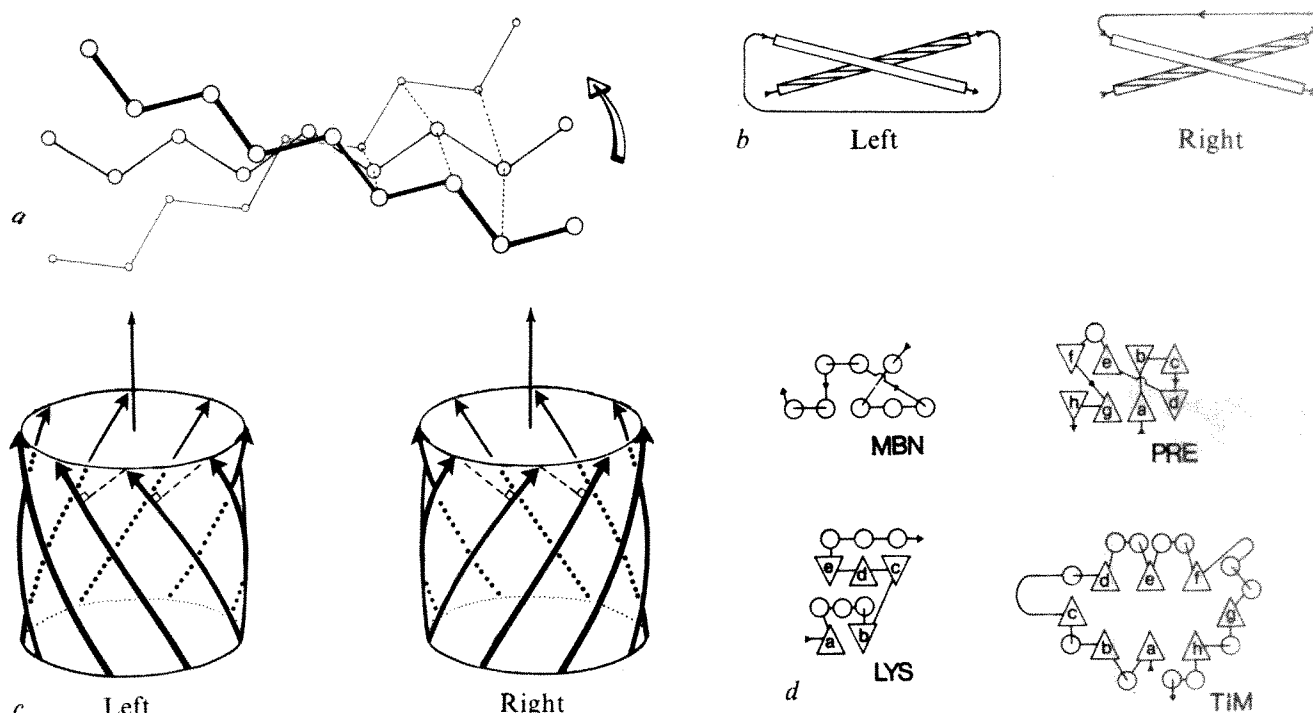
to be buried inside the protein forming a non-polar core, while hydrophilic side chains are nearly always exposed³³. Nearly all the buried polar groups, such as the main-chain $-\text{NH}$ and $-\text{CO}$ groups form hydrogen bonds³⁴. Consequently chain reversals almost always occur near the surface of the molecule³⁵ while large parts of the α -helices and β -sheets are buried. Any realistic prediction must aim to satisfy both these requirements.

Further restrictions on possible conformations occur because of the chirality of L-amino acids. In particular: (1) α -helices are right-handed. (2) β -sheets have a right-handed twist of the peptide units along the β -strand and, consequently, a left-handed rotation between adjacent strands³⁶ (Fig. 2a). (3) The connection between two parallel strands in the same sheet will almost certainly be right-handed (Fig. 2b)³⁷⁻³⁹. (4) Cylindrical sheets (that is β -barrels) are right-handed (Fig. 2c)⁴⁰. The first requirement for right-handed α -helices can be incorporated into a conventional energy minimisation (see later), but the remaining observations refer more specifically to tertiary structure and suggest that a model-building approach to protein prediction could be useful.

One of the main difficulties in predicting tertiary structure is to determine which segments of chain will lie close together. It has been observed that elements of secondary structure which are sequential along the polypeptide chain tend to lie proximate in the three-dimensional structure⁴¹. Furthermore analyses of β -sheets have suggested several preferences which help determine strand order^{42,43}. For example, the last sequential strand in the sheet preferentially occupies an edge position. Similarly the most hydrophobic strands generally occupy central positions in the sheet, with the more hydrophilic strands at the edge⁴⁴. These preferences should also be incorporated into prediction schemes (see later).

Several features of the overall fold have been recognised. Certain tertiary structures of more than an individual α -helix or β -sheet are often observed and appear to act as 'building blocks' or 'super-secondary structures'⁴⁵. Large proteins are often divided into a few distinct structural regions, called domains. These

Fig. 2 Results of analysis of protein structure. *a*, A twisted β -sheet: schematic diagram of three β -strands viewed normal to their direction to show the left-handed rotation between them that is always observed³⁶. *b*, Two parallel β -strands, viewed normal to their direction, and a connecting region. The right-handed structure is nearly always observed whereas the left-handed structure occurs rarely³⁷⁻³⁹. This may be because the left-handed rotation between β -strands gives a direct connection for the right-handed structure³⁷. *c*, The handedness of the β -barrel. In all observed barrels the strands are inclined to the axis so as to form a right-handed structure⁴⁰, that is if the strands were extended they would tend to form a right-handed helix about the barrel axis. This handedness is a consequence of the twist of the β -sheet. The alternative left-handed structure has not been observed. *d*, Classes of protein structure⁴¹. MBN-myoglobin, an α -protein; PRE-prealbumin, a β -protein; LYS-hen egg white lysozyme, an $\alpha + \beta$ protein; TIM-triose phosphate isomerase, an α/β protein.



domains contain 40–250 residues. Finally most protein structures fall into five rather distinct classes⁴¹ which are defined according to secondary structure content (Fig. 2d): α -proteins have only α -helix; β proteins have mainly β -sheet; $\alpha + \beta$ proteins have α -helix and β -strand secondary structural segments that do not mix but tend to segregate along the polypeptide chain; α/β have mixed or approximately alternating segments of α -helix and β -strand; and 'coil' proteins contain almost no regular secondary structure. We have noted that intracellular proteins usually fall into the α or α/β classes, and almost never have disulphide bridges. In contrast, extracellular proteins are usually all β or $\alpha + \beta$ type.

Once a protein sequence is known and a secondary structure prediction has been performed, these observations will help to predict the correct tertiary structure. Although some of these results have already been used, most of this work has yet to make its impact on prediction.

Tertiary structure prediction from evolutionary relationships

Families of homologous proteins, for example the cytochromes c, have almost identical main-chain structures but only similar sequences, suggesting that structure evolves more slowly than sequence⁴⁶. Observed structures can therefore be used to predict unknown structures of proteins with related sequences. For example, the fold of α -lactalbumin has been correctly predicted on the basis of its sequence similarity with hen egg white lysozyme⁴⁷. Similarly, a structure for troponin C was predicted from the crystal structure of myogen in conjunction with symmetry requirements⁴⁸. Recently, sequences that show very little similarity have been observed to adopt similar fold, for example in the case of the dehydrogenases⁴⁹. This has proved useful for prediction, although it is debatable whether these similarities result from convergent or divergent evolution. For example, parts of aldolase⁵⁰ and glutamate dehydrogenase⁵¹ have been predicted to fold in a similar six-stranded structure to lactate dehydrogenase. This is based on secondary structure prediction, sequence resemblances with the dehydrogenases and, for aldolase, recognition of the fold by affinity chromatography⁵⁰.

One can envisage that grouping proteins into families may be used widely to predict structure. Furthermore many proteins have evolved by gene replication or gene fusion, and the structural consequences of this may also be useful for prediction. However, this type of approach is necessarily restricted to related proteins.

Prediction of tertiary structure

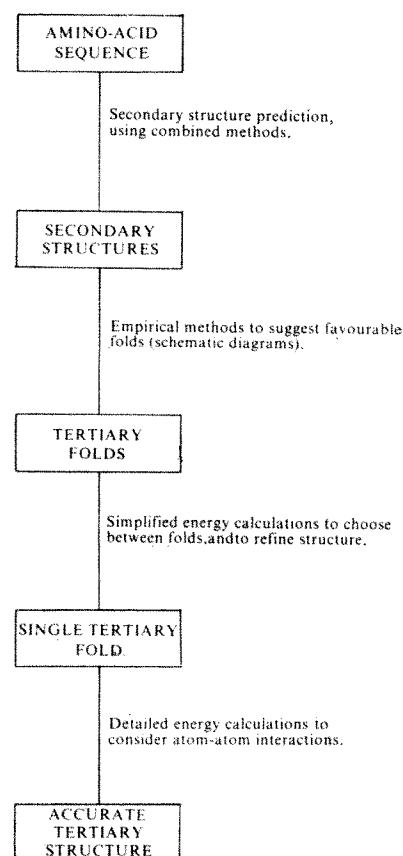
Predictions of the tertiary structure of a protein by more general methods have been made only very recently. At this early stage the predictions have been limited to small proteins whose structures are already known. There are several completely different approaches.

The semi-empirical energy calculations described previously have been modified to treat proteins by a simplification of energy functions and protein structure representation (Fig. 1e), followed by energy minimisation^{52–55}. These methods are based on the assumptions that: (1) the fine details of protein structure can be time-averaged and are not vital for the formation of the correct fold of the polypeptide chain; (2) only variables that have the greatest effect on folding need be considered; (3) the major determinants of the protein fold are the requirement for close-packing (van der Waals interactions), good hydrophobic burial and hydrophilic exposure (solvent interactions) and sometimes the ϕ - ψ preference of certain amino acid types. Probably the choice of these determinants was influenced by the analyses of known structures. These simplifications were introduced by Levitt and Warshel⁵². They represented each amino acid residue by two centres, the C α atom and a single sphere positioned at the centroid of the side chain (Fig. 1e). The interactions were between these centres. Only one degree of freedom—the torsion angle α about a line joining consecutive C α atoms—was allowed per residue⁵⁵. Minimisation of the energy function was followed by 'thermalisation' to escape from local minima. The method was

applied to pancreatic trypsin inhibitor (58 residues with one α -helix of ten residues) and myogen (108 residues with six α -helices). Generally residues that formed α -helices were preset to their correct conformation and held rigid during the calculation. A similar algorithm has been developed by Kuntz *et al.*⁵³ who minimised an error function which incorporated hydrophobic/hydrophilic interactions, steric repulsion and correct disulphide and iron-sulphur bonding. The structure of trypsin inhibitor and rubredoxin (53 residues) were predicted. Tanaka and Scheraga⁵⁴ have outlined a third method which shares some features with the two approaches described above. Each side chain is represented by a single sphere and the interactions between pairs of residues is estimated from an analysis of close contacts in 25 proteins of known structure. The secondary structure for each residue was predicted. Low energy conformations were found by a random 'Monte-Carlo' search, rather than by minimisation. The method was applied to trypsin inhibitor.

The success of these methods can be measured as the r.m.s. deviation of the predicted structure coordinates from the observed structure. Levitt and Warshel obtained r.m.s. errors of 5–7 Å for trypsin inhibitor. Conformations that deviated more from the native structure always had higher energies, which gives an independent criterion for choosing the best conformation. They had less success with myogen, obtaining only three structures with r.m.s. values less than 8.5 Å, even assuming rigid helices. Similarly Kuntz *et al.* obtained a number of structures for each protein with r.m.s. errors of 4 to 6.5 Å for trypsin inhibitor and rubredoxin (note: the disulphide and sulphur-iron bonding was forced to be correct). In both methods some, though by no means all, of the minimised structures had the correct fold, with bends and loops approximately located and the correct long-range interactions. Myogen was the most noticeable exception, since even the structures with lowest r.m.s. errors showed an incorrect fold. Tanaka and Scheraga do not give r.m.s. values but claim to have obtained some agreement with the native structure.

Fig. 3 A possible approach to predicting tertiary structure from amino acid sequence using a combination of methods.



Several serious points arise from consideration of these results. These methods have not yet been sufficiently tested for it to be clear whether they will be generally useful and whether the assumptions made are valid. Their application to larger proteins is difficult to envisage since the computing time will increase rapidly and larger structures are more complex. Levitt (personal communication) has suggested that compact structures of trypsin inhibitor, generated by a self-avoiding random walk, are almost as close to the native structure as some of the better structures obtained from energy minimisation. Thus it is questionable whether the simple structural representation and energy minimisation techniques are adequate. Finally, it is difficult to see how many of the empirical observations on tertiary structure can be incorporated into this kind of approach.

Overall, the results of these simplified energy calculations were very encouraging. Starting from a fully extended conformation, proteins have been folded into compact structures which show many similarities to the native states. It is to be hoped that these methods will be more fully explored and refined to give improved predictions.

A different type of approach to predict the structure of α -helical proteins has been described by Ptitsyn and Rashin⁵⁶, used by Lim and Efimov⁵⁷, and modified for application to β -sheet proteins by Zav'yalov⁵⁸. There are two basic assumptions. First, folding proceeds by a stepwise process in which fluctuating regions of secondary structure are formed and then condense into a single compact globule. Second, the condensation process is determined by three considerations: (1) Regions of secondary structure, which are adjacent along the polypeptide chain, will tend to lie proximate in the tertiary structure. (2) At each stage of the folding pathway only the most favourable structures are maintained and all other structures rejected. (3) Packing will occur so as to maximise the reduction of hydrophobic free energy, by the exposure of hydrophilic groups and burial of hydrophobic side chains.

Thus Ptitsyn and Rashin⁵⁶ took the residues forming α -helices in myoglobin from the native structure and modelled these α -helices by cylinders. A manual search was performed for the packing between adjacent helices which gave optimal reduction in hydrophobic free energy. They were able to predict the correct native fold. Similarly Lim and Efimov have folded tobacco mosaic virus protein. Zav'yalov⁵⁸, assuming the hairpin bends in the immunoglobulin domain, predicted the structure of the β -sheet sandwich. The success of this approach indicates the importance of hydrophobic free energy and close-packing of secondary structures in determining tertiary structure. The major problem is that the method is non-automated and therefore difficult to apply impartially. However a computerised method based on this approach, which incorporates the packing rules for secondary structures³² and includes some calculation of hydrophobic free energy could prove successful.

Most recently a different approach has been considered in which, rather than energy calculations, folding rules obtained from the analyses of structures are used for prediction. We have used the results from a quantitative analysis of β -sheets to derive a folding parameter which represents whether a particular arrangement of strands in a sheet is favourable⁵⁹. This approach involves a further level of simplification: the calculations only consider the relative positions and type of connections of the strands (as in Fig. 1c) and do not evaluate residue-residue interactions. To explore this approach, the location of the secondary structure along the chain and the assignment of strands to a particular sheet were taken from the observed structure. For 25 out of the 52 sheets examined, the actual arrangement is the sheet, or one of the sheets, with the most favourable folding parameter and therefore could have been readily predicted. This surprisingly good result suggests that strand order is somewhat determined by a few elementary folding rules.

Nagano (personal communication) has suggested another empirical approach to predicting probable packing arrangements of α -helices and β -strands in α/β type proteins. First, regions of the sequence that are likely to be predicted as α or β secondary

structures are located. Then hypothetical rules are used to suggest packing arrangements whose stability is evaluated by an empirical penalty function. Thus he builds up a schematic diagram of the protein structure (like that in Fig. 1c). The generality of this method has still to be determined. However it is a novel approach, which has the advantage of starting from the sequence and translating right through to the tertiary structure.

These empirical methods, by their simplicity, can be applied to larger proteins whose size puts them beyond the scope of conventional methods. The approaches consider the structural features which result from both the stability of the final structure and the kinetics of protein folding, without any assumptions about the folding process. They should be useful to predict probable folds which then can be examined by energy calculations.

Outlook

Although the individual methods of structure prediction will doubtless be extended and improved, it is likely that the answer to the folding problem lies in a combination of the available approaches. One combination is outlined in Fig. 3. This shows how, starting with the sequence, one can progress through the secondary structure analysis and the prediction of tertiary fold to simplified and then detailed energy calculations. Feedback at each stage is important, so that, for example, the final choice of secondary structure must be influenced by consideration of which tertiary folds are favourable. For an individual protein, biochemical data (for example, which residues are involved in the active site) can be incorporated, either to suggest which of the tertiary folds should be chosen, or to test the feasibility of the final predicted structure. Perhaps the major improvements to prediction will come from further renaturation and X-ray studies, which will provide more information on folding pathways and the final structure. Clearly the last few years have witnessed the development of many novel approaches to the prediction of tertiary structure. Hopefully these preliminary investigations will provide the first steps towards the solution of the folding problem.

We thank: Professor D. C. Phillips for encouragement and advice; Dr I. A. Wilson for help in preparation of the stereo diagram; Dr Olga Kennard for supplying coordinates of lactate dehydrogenase from the Cambridge Data Bank; Professor M. G. Rossmann and Academic Press, New York, for allowing us to reproduce Fig. 7 from reference 49; and the Medical Research Council for financial support.

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articles

SU(2) × U(1): A gauge theory of weak interactions?

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We construct a gauge theory of weak interactions which fits all neutral current data, including the atomic physics experiments. It is shown that it is possible to include this theory in a unified theory of weak and electromagnetic interactions; the desirability of doing so is discussed.

IN 1967 Weinberg¹ wrote down a renormalisable unified gauge theory of weak and electromagnetic interactions based on the spontaneously broken gauge group SU(2) × U(1). This model involves the existence of a weak neutral current coupled to neutrinos, and weak neutral currents were discovered experimentally² in neutrino scattering in 1973. So it has been a great temptation for physicists, especially theorists, to claim that the discovery of neutral currents is a confirmation of the Weinberg model and in particular shows the unification of weak and electromagnetic interactions.

This view although widespread has been forcefully criticised, especially by Sakurai³. He argues that neutral current phenomena are interesting in their own right, and that it is the job of physicists to discover through experiment the relevant couplings not to assume the results in advance. Nevertheless the Weinberg model has been very successful and all neutrino data up to now are consistent with a single value of $\sin^2\theta_w$. The model is obviously useful as it not only provides a means of analysing experiments but it also suggests new experiments. In Bjorken's words⁴ it is a "working hypothesis". In particular in its simplest form it gives definite predictions of parity violation in heavy atoms which were not observed in recent experiments at Oxford and Washington⁵.

The purpose of this article is to point out another possibility. The starting point is a remark made some years ago⁶: the requirement that the theory of weak interactions is renormalisable has nothing *a priori* to do with unification of weak and electromagnetic interactions. After all it is (or at least was) customary to treat strong, electromagnetic and weak interactions independently of each other.

We shall treat this remark perhaps more seriously than it deserves in order to construct a new working hypothesis. We shall write down a SU(2) × U(1) gauge theory of weak interactions which as far as neutrino interactions are concerned allows an almost identical analysis of the present data. This theory gives parity violation in heavy atoms but the magnitude of the effect predicted is now in agreement with experiment. The theory is renormalisable in the absence of electromagnetic and strong interactions.

We shall construct our weak SU(2) × U(1) model first and discuss its difficulties afterwards. We start with the leptonic

sector. Following Weinberg we take a doublet $L = (\nu, e)_L$ of left-handed leptons and a right-handed lepton singlet $R = e_R$ with a Lagrangian

$$\mathcal{L} = i\bar{L}\gamma^\mu(\partial_\mu + \frac{1}{2}ig\tau\cdot\mathbf{W}_\mu - \frac{1}{2}ig'B_\mu)L + i\bar{R}\gamma_\mu(\partial_\mu - irB_\mu)R + \mathcal{P} \quad (1)$$

where \mathcal{P} involves the meson-meson couplings required by gauge invariance.

Take

$$\begin{aligned} Z_\mu &= W_\mu^3 \cos\theta_w - B_\mu \sin\theta_w \\ X_\mu &= W_\mu^3 \sin\theta_w + B_\mu \cos\theta_w. \end{aligned} \quad (2)$$

In the unified theory X^0 is the photon and the constraints are (1) no $\bar{\nu}\nu X^0$ coupling and (2) the $\bar{e}eX^0$ coupling is pure vector. These conditions give $g' = g \tan\theta_w$ and $r = g'$. This leads to a Lagrangian

$$\begin{aligned} \mathcal{L} &= \mathcal{L}_0 - \frac{g}{2\sqrt{2}}[\bar{\nu}\gamma^\alpha(1-\gamma_5)eW_\alpha^+ + h.c.] \\ &- \frac{g'}{4\sin\theta_w}Z^\alpha[\bar{\nu}\gamma_\alpha(1-\gamma_5)\nu - (1-4\sin^2\theta_w)\bar{e}\gamma_\alpha e + \bar{e}\gamma_\alpha\gamma_5 e] \\ &+ g\sin\theta_w X^\alpha \bar{e}\gamma_\alpha e + \mathcal{P}. \end{aligned} \quad (3)$$

In weak SU(2) × U(1) X^0 is not the photon but a second neutral vector meson. The requirement that there should be the same relation between neutrino scattering by charged currents and neutral currents in this model as in the unified theory implies again that there is no $\bar{\nu}\nu X^0$ coupling. So again $g' = g \tan\theta_w$. In order to obtain a distinct alternative theory we require now that the $\bar{e}eX^0$ coupling is pure axial. This gives $r = -g'$. With these choices the Lagrangian becomes

$$\begin{aligned} \mathcal{L} &= \mathcal{L}_0 - \frac{g}{2\sqrt{2}}[\bar{\nu}\gamma^\alpha(1-\gamma_5)eW_\alpha^+ + h.c.] \\ &- \frac{g'}{4\sin\theta_w}Z^\alpha[\bar{\nu}\gamma_\alpha(1-\gamma_5)\nu + (1-4\sin^2\theta_w)\bar{e}\gamma_\alpha\gamma_5 e - \bar{e}\gamma_\alpha e] \\ &- g\sin\theta_w X^\alpha \bar{e}\gamma_\alpha\gamma_5 e + \mathcal{P}. \end{aligned} \quad (4)$$

Comparing the Lagrangians (3) and (4) we see that there is no difference in neutrino couplings but that in the $\bar{e}eZ^0$ couplings the vector and axial currents are interchanged.

The GIM⁷ mechanism can now be used as in the unified theory to construct the weak hadronic interaction. We will not display

the result; all we need is that whereas in unified $SU(2) \times U(1)$ the $\Delta S = \Delta C = 0$ neutral weak hadronic current is

$$J_\mu = A_\mu^3 + (1 - 2\sin^2\theta_w)V_\mu^3 - 2\sin^2\theta_w A_\mu^S, \quad (5)$$

in weak $SU(2) \times U(1)$ the current coupling to Z^0 is

$$J_\mu = V_\mu^3 + (1 - 2\sin^2\theta_w)A_\mu^3 - 2\sin^2\theta_w A_\mu^S, \quad (6)$$

where V_μ and A_μ are the vector and axial vector currents, and 3 and S refer to the isovector and isoscalar components. There is also a purely axial current which couples to X which will not contribute to neutrino scattering.

Before turning to the neutral current data, we must define the strength of neutral current neutrino processes compared with charged current processes in the effective current theory. The Fermi constant G for charged currents is given in terms of the W^\pm mass m_w by

$$\frac{G}{\sqrt{2}} = \frac{g^2}{8m_w^2}. \quad (7)$$

Similarly, if the Fermi constant for neutral currents is G' , then from equation (4)

$$\frac{G'}{\sqrt{2}} = \frac{g'^2}{16m_z^2\sin^2\theta_w} = \frac{g^2}{16m_w^2\kappa^2} \quad (8)$$

where $\kappa = m_z \cos\theta_w / m_w$. In the Weinberg model $\kappa = 1$. (Note that Salam and Ward⁸, following Glashow⁹, wrote down an $SU(2) \times U(1)$ unified gauge theory in 1964. As it was not a spontaneously broken gauge theory, however, it was not renormalisable and there were no predictions of the 'vector mesons' masses. Thus the structure of their weak neutral current is that given by Weinberg, but the relative strength of the neutral current compared with the charged current in neutrino interactions is not specified. Only with a detailed symmetry breaking mechanism can κ be predicted and in our opinion this is the distinguishing feature of Weinberg's model.)

Experimentally $\kappa^2 = 0.97 \pm 0.13$ (ref. 10). In weak $SU(2) \times U(1)$ we will show shortly that $\kappa < 1$, but for the moment we assume that the value predicted is in agreement with experiment. We can now analyse the neutral current data.

Inclusive neutrino reactions

The well-known vector-axial ambiguity¹¹ implies that the results in our model are identical with those of the unified $SU(2) \times U(1)$ model. Thus we can use the Weinberg angle measured in these experiments. The most recent values¹² are $\sin^2\theta_w = 0.31 \pm 0.03$ or $\sin^2\theta_w = 0.26 \pm 0.02$, depending on whether the CDHS (CERN-Dortmund-Heidelberg-Saclay) data is included. We shall be conservative and take $0.24 \leq \sin^2\theta_w \leq 0.34$.

Neutrino-lepton scattering

Again, there is no effect when vector and axial currents are interchanged. So the results of unified $SU(2) \times U(1)$ model are true in weak $SU(2) \times U(1)$.

Elastic νp scattering

The cross section in exclusive channels is not invariant under the interchange of vector and axial vector currents. For example, in the forward direction we have in the Weinberg model from equation (5)

$$R = \lim_{q^2 \rightarrow 0} \left[\frac{d\sigma}{dq^2}(\nu p \rightarrow \nu p) / \frac{d\sigma}{dq^2}(\nu n \rightarrow \mu^- p) \right] \\ = \frac{1}{4}[(1 - 4\sin^2\theta_w)^2 + (g_A^3)^2] / [1 + (g_A^3)^2], \quad (9)$$

where $g_A^3 = -1.25$ is the isovector axial renormalisation factor. In weak $SU(2) \times U(1)$ we have from equation (6)

$$R = \frac{1}{4\kappa^2} [1 + \{(1 - 2\sin^2\theta_w)g_A^3 - 2\sin^2\theta_w g_A^S\}^2 / [1 + (g_A^3)^2]]. \quad (10)$$

The isoscalar axial renormalisation factor g_A^S is not experimentally known, but a reasonably reliable quark model estimate¹³ is $g_A^S = \frac{2}{3}g_A^3$. Putting in the values for the parameters we obtain in the Weinberg model $0.15 < R < 0.17$, whereas in weak $SU(2) \times U(1)$ $R = 0.10/\kappa^2$ and the dependence on g_A^S is not very sensitive.

The data are not yet good enough to determine more than total cross sections integrated over all q^2 . For this assumptions must be made about axial form factor behaviour which are not well known, and it is safe to say that any conclusion drawn on the basis of the Weinberg model can be matched in weak $SU(2) \times U(1)$.

Single pion production

This process is very difficult to analyse as most of the data come from heavy liquid bubble chambers where nuclear charge exchange effects are important. There is data from Argonne¹⁴ in hydrogen and deuterium but the errors are still very large. So again it is unlikely that any prediction could be made on the basis of unified $SU(2) \times U(1)$ which could not be reproduced in weak $SU(2) \times U(1)$.

Comparing equations (5) and (6) we see that A_μ^3 is suppressed in weak $SU(2) \times U(1)$ compared with in the Weinberg model, whereas V_μ^3 is enhanced. As A_μ^3 gives the dominant transition in the Δ -resonance region, a calculation of the cross section for a given value of $\sin^2\theta_w$ would give a lower value in weak $SU(2) \times U(1)$ than in unified $SU(2) \times U(1)$. The theoretical analysis¹⁵ of weak pion production in unified $SU(2) \times U(1)$ shows that cross sections decrease as $\sin^2\theta_w$ increases. Hence if weak $SU(2) \times U(1)$ is right an analysis of this process in unified $SU(2) \times U(1)$ would give a relatively large value of $\sin^2\theta_w$.

Parity violation in atoms

Z^0 exchange between an atomic electron and the nucleus will not conserve parity. This effect arises both from a vector interaction at the electron together with an axial interaction at the nucleus, and from an axial interaction at the electron together with a vector interaction at the nucleus. Taking the nucleus as static the latter term will dominate in heavy nuclei as it gives a coherent contribution over the whole nucleus. In the Weinberg model the coefficient of the axial coupling is taken as unity from equation (3) whereas the nuclear vector contribution from equation (5) gives an overall effect

$$Q_w = (1 - 4\sin^2\theta_w)Z - N. \quad (11)$$

Numerically in bismuth $123 < -Q_w < 156$. From the experiment⁵, the observed value of Q_w in bismuth is no larger than one-tenth of the maximum value predicted by the Weinberg model together with the standard atomic theory. The standard atomic theory should be good to at least a factor two¹⁶, so it is fair to say that $|Q_w| < 32$ for bismuth.

In weak $SU(2) \times U(1)$ we have a factor $1 - 4\sin^2\theta_w$ for the axial coupling from equation (4), and the nuclear vector coupling is pure isovector from equation (6). The exchange of X^0 conserves parity. So now

$$Q_w = (1 - 4\sin^2\theta_w)(Z - N)/\kappa^2 \quad (12)$$

or in bismuth $-2 < Q_w\kappa^2 < 15$, which is not in disagreement with the experiments.

If $\sin^2\theta_w = \frac{1}{4}$ then $Q_w = 0$, as the $\bar{e}eX^0$ coupling is now pure vector. This would rule out any coherent effect although there would still be parity violation from the axial coupling of the nucleus. This would be seen most clearly in experiments on

hydrogen or deuterium or in the experiment to measure a macroscopic electric dipole moment in superfluid Helium 3 suggested by Leggett¹⁷. It is also worth noting that for $\sin^2\theta_w = \frac{1}{4}$ in weak $SU(2) \times U(1)$ there is no parity violation in purely leptonic neutral current interactions. So there is no forward-backward asymmetry in $e^+e^- \rightarrow \mu^+\mu^-$ and $\sigma(\nu_\mu e^-) = \sigma(\bar{\nu}_\mu e^-)$.

We now turn to the mass relations of the gauge particles in weak $SU(2) \times U(1)$. We require spontaneous breaking with respect to $SU(2)$ through a single isospinor complex scalar field as in the Weinberg theory, but now we also spontaneously break the $U(1)$ through an isoscalar complex scalar field, as the current coupled to the X^0 is no longer conserved. Thus two Higgs scalars result and the particles W^\pm , X^0 , Z^0 acquire mass. The mass relations follow from the mixing equations

$$\begin{aligned} m_x^2 &= m_w^2 \sin^2\theta_w + m_B^2 \cos^2\theta_w + 2m_{wB}^2 \sin\theta_w \cos\theta_w \\ m_z^2 &= m_w^2 \cos^2\theta_w + m_B^2 \sin^2\theta_w - 2m_{wB}^2 \sin\theta_w \cos\theta_w \\ 0 &= m_{wB}^2 (\cos^2\theta_w - \sin^2\theta_w) + (m_w^2 - m_B^2) \sin\theta_w \cos\theta_w \end{aligned} \quad (13)$$

where m_{wB}^2 is the off-diagonal element of the mass matrix. So

$$m_w^2 = m_z^2 \cos^2\theta_w + m_x^2 \sin^2\theta_w. \quad (14)$$

For $m_x = 0$ this reduces to $m_w = m_z \cos\theta_w$ and $\kappa = 1$, as in the Weinberg model. Now, however, we are constrained by the experimental value of κ . Since $\kappa \sim 1$, m_x cannot be too large. Nevertheless, since the data allow $\kappa^2 > 0.84$, the previously given bounds on $\sin^2\theta_w$ enable us to deduce from equation (14) that $m_x/m_w < 0.7-0.8$.

Note that there is no difficulty in giving mass to X^0 , so it cannot be claimed that a photon is a necessary concomitant of the theory. If the gauge group were simple, as in the Georgi-Glashow model¹⁸, and if the symmetry breaking were through the regular representation¹⁹, then it would be natural to have a zero mass vector meson in the theory: theories of this type are discussed elsewhere²⁰. In weak $SU(2) \times U(1)$ the value of m_w is not constrained. In unified $SU(2) \times U(1)$ $g \sin\theta_w = e$ and using equation (7) this fixes the mass scale of the theory so that $m_w > 37$ GeV. In weak $SU(2) \times U(1)$ g is just the weak coupling constant and is arbitrary. Indeed this model is flexible: if no vector mesons are found then the limit $g \rightarrow \infty$, $m_w \rightarrow \infty$ can be taken and the old four-fermion theory reappears.

It is now time to mention the difficulty of a gauge theory of weak interactions which leaves out electromagnetism. The process $e^+e^- \rightarrow W^+W^-$ through one photon exchange displays all the old problems associated with charged spin on particles. In general the electromagnetic corrections to the theory are not renormalisable.

Of course the obvious way out of this difficulty is to reunify the theory after all by including the photon as a gauge particle and enlarging the group. The minimal extension of weak $SU(2) \times U(1)$ is $SU(2) \times U(1) \times U(1)$, where the new $U(1)$ is characterised by another isoscalar vector meson C_μ and the photon field

$$a_\mu = W_\mu^3 \sin\psi + C_\mu \cos\psi. \quad (15)$$

Then, as the resultant theory is unified, $e = g \sin\psi$, and immediately we obtain the unified mass scale $m_w = 37/|\sin\psi|$ GeV. Provided $\sin\psi$ is reasonably small all the results of weak $SU(2) \times U(1)$ will still hold, up to an error of order $|\sin\psi|$. There is now no connection between $\sin\theta_w$ determined from neutrino scattering and m_w . Equation (14) still holds approximately and so although W^\pm must now have a large mass X^0 can still be light²¹.

The historic reasons^{8,9} for a non-Abelian gauge theory of weak interactions are that these involve charged currents which are conserved—the natural way of ensuring this is to construct a gauge theory. Alternative possibilities to a unified theory of weak and electromagnetic interactions are suggested by analogy with strong interaction theory where there were also good reasons for writing a non-Abelian gauge theory in order to explain isotopic spin conservation. Sakurai²² introduced the ρ meson as a gauge particle for this purpose. Gell-Mann²³ constructed the $SU(3)$

symmetry among interactions by generalising these ideas. Yet these theories involved charged vector mesons ρ^\pm and therefore were open to the same criticism as here: these theories are non-renormalisable in the presence of electromagnetism.

These criticisms are no longer relevant. The modern picture of ρ^\pm is a composite of two spin- $\frac{1}{2}$ particles, a quark-antiquark pair. The ultra-high energy behaviour of $e^+e^- \rightarrow W^+W^-$ similarly depends on the internal electromagnetic structure of W^\pm . Form factors arising from a composite nature (a pair of quarks, heavy leptons, leptoquarks?) or a hidden strong interaction of W^\pm could save the day. So could a reinterpretation of quantum electrodynamics for high spin particles along the lines suggested by Lee and Yang².

All gauge theories require the existence of intermediate vector mesons. In a unified gauge theory the mass scale is fixed: $m_w \sim e/G^{1/2} \approx 38$ GeV. In a weak gauge theory this is not necessarily so, and, if weak interactions were really weak at all energies, we would expect relatively light W^\pm , for example if $g^2/4\pi = 10^{-3}$, then $m_w = 14$ GeV. Experimentally the lower limit on m_w is 1 GeV (D. H. Perkins, personal communication).

In weak $SU(2) \times U(1)$ a neutral vector meson X^0 exists which must be lighter than W^\pm . It has not yet been observed in e^+e^- colliding beam experiments at SPEAR, so $m_x > 7.6$ GeV (ref. 25). It could be identified by its (relatively) narrow width

$$\Gamma = (2+R) \frac{G^2/2}{3\pi} m_x^2 \sin^2\theta_w \quad (16)$$

where in this model R is the cross section ratio $\sigma(e^+e^- \rightarrow \text{hadrons})/\sigma(e^+e^- \rightarrow \mu^+\mu^-)$ at centre-of mass energy m_x . So if $m_x = 10$ GeV and $m_w = 90$ GeV, then $\Gamma \sim 250$ MeV.

We should emphasise that even if the theory is unified, so that $SU(2) \times U(1) \times U(1)$ or a larger group such as $SU(2) \times SU(2) \times U(1)$ (refs 26, 27) or $SU(3) \times U(1)$ (ref. 28) is applicable, it may still be useful to consider weak $SU(2) \times U(1)$ as an approximate symmetry for weak interactions. Indeed the Weinberg model is perhaps too constrained a theory. In weak $SU(2) \times U(1)$ $\sin\theta_w$ fixes the form of the neutral currents but it does not fix the strength relative to the charged current; for that κ is needed, where $\kappa \neq 1$. Again, in the Weinberg model the neutral vector meson must be heavier than W^\pm , while in weak $SU(2) \times U(1)$ the X^0 can be arbitrarily light. Only if and when the vector mesons are found can definite statements be made about the underlying theory. It follows that experimentalists should continue to look for W^\pm and neutral weak vector mesons, and not assume that the success of the Weinberg model implies that they can only be found with a new generation of accelerators.

Finally we point out that there is one good (if not compelling) reason for the unification of weak and electromagnetic interactions which results from experiment. This comes about by inverting the argument of Wilkinson²⁹ on the radiative corrections to vector current universality. If the observed muon decay rate is compared with nuclear ft-values, it is found that

$$\Delta \equiv 2\{g_{\beta\nu}^R/g_\mu^R \cos\theta_c - 1\} = 2.19 \pm 0.27\% \quad (17)$$

where $g_{\beta\nu}^R$ and g_μ^R are the effective β decay and muon decay coupling constants (after removing the well-understood 'outer' radiative corrections) and θ_c is the Cabibbo angle. Theoretically the purely electromagnetic contribution to Δ , arising from the 'inner' (short distance) radiative corrections gives a divergent contribution³⁰

$$\Delta = \frac{3\alpha}{2\pi} (1 + 2\bar{Q})(\ln(\Lambda/m_N)) \quad (18)$$

where Λ is a cut-off and \bar{Q} is the average charge of the quark constituents of the nucleon. Assuming $\bar{Q} = \frac{1}{6}$, as is appropriate for both the conventional Gell-Mann Zweig quarks used throughout this analysis and integrally charged Han Nambu quarks³¹, we obtain from equations (16) and (17) that $\Lambda \approx 90$ GeV.

In a unified theory of weak and electromagnetic interactions the average mass of the gauge vector mesons would provide an

effective cut-off³²⁻³⁴. So we conclude that this result implies that the value of m_w is about 90 GeV (ref. 35), which is consistent with the mass scale expected in a unified theory.

We thank Roger Blin-Stoyle, Alex Love, Don Perkins and Patrick Sandars for discussions.

Received 9 October; accepted 30 October.

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Granite-greenstone terrain Rhodesian Archaean craton

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Granite-greenstone terrains of possibly three different ages exist in the Rhodesian Archaean craton. Their respective greenstone belts can be tentatively delineated across the central cratonic area. The main stratigraphic units of the youngest (~ 2,700 Myr) and most widespread development can be correlated across greenstone belts in this area. Major events in the craton are summarised and briefly discussed in the light of the new correlations and recent age determinations.

A REVIEW¹ of available field and geochronological data allows the recognition and partial delineation of granite-greenstone terrains of different ages within the Basement Complex of the Rhodesian Archaean craton. The oldest terrain is ~ 3,500 Myr old and occurs in the southern and central parts (Fig. 1 and Table 1). Its greenstone belt remnants equate largely with the Sebakwian Group of the Rhodesia Geological Survey as delineated by Wiles². The main greenstone belts are younger and can be divided into the widespread Upper Greenstones and the more problematical Lower Greenstones which together correspond largely to the Bulawayan Group of Wiles. The Sebakwian and Bulawayan Groups as delineated by Anhaeusser³, on the basis of the Barberton model, do not correspond to those of Wiles, or to the subdivisions recognised in this paper.

The age of the Lower Greenstones is obscure. They were deformed and eroded before the deposition of the Upper Greenstones. The results obtained so far on Lower Greenstones material yield ages in the ~ 2,700 Myr bracket, but initial ⁸⁷Sr/⁸⁶Sr ratios are higher than the near 0.701 values typical of the Upper Greenstones. Whether such high initial ratios (for example 0.7056 ± 0.002, Hokonui tuffs⁴) merely reflect the primary composition of these rocks, or can be taken as evidence for metamorphic rotation of the isochron to an age significantly younger than their age of formation, is still debatable. On

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ands¹ it is possible that the Lower Greenstones are granite-greenstone terrain incorporating tonalites and gneisses. For example the Mashaba tonalite and Chingezi gneisses of Belingwe) dated at ~ 2,900 Myr (Table 1; refs 4,5). Greenstones are ~ 2,700-Myr-old and the low initial ratios are consistent with this being an age of eruption⁶. To the east and the west the Upper Greenstones are overlain by the Bulawayan Group. Condie⁷ have recently offered an age of 3,080 ± 60 (errors) for the Bulawayo-Que Que greenstone belt, but might place any geological significance on this result. Geologically their data points involve a spread of 200 km and lithically they range from Lower Greenstones to the top of the Upper Greenstones. Moreover their selection of 15 points from the 50 considered, seems somewhat arbitrary.

The 3,500 Myr terrain

The development of ~ 3,500-Myr-old rocks occurs within a large triangular crustal segment with Selukwe, Fort Victoria and Anabani near the approximate corners⁸. Much of this segment consists of gneisses such as those around Shabani, and the gneisses west of Mashaba (Table 1). Most are tonalitic and highly deformed and, together with the infolded greenstone belt remnants, look like the remains of an ancient, northerly trending, mobile belt. Between Mashaba and Fort Victoria the gneisses are cut by the ~ 3,500-Myr-old Mushandike granite^{9,10} and elsewhere by younger granitic rocks¹. The greenstone belt remnants within the segment, and the presumed ~ 3,500 Myr-old remnants elsewhere in the central cratonic area (Fig. 1), consist of various sedimentary and igneous metasedimentary rocks and other mafic and ultra-mafic types. Felsic metavolcanic rocks are apparently absent, although they would be difficult to recognise within the gneisses. Outside the triangular segment it is not clear how much of the associated gneissic terrain is ~ 3,500 Myr old. The best documented of the larger areas of ~ 3,500-Myr-old

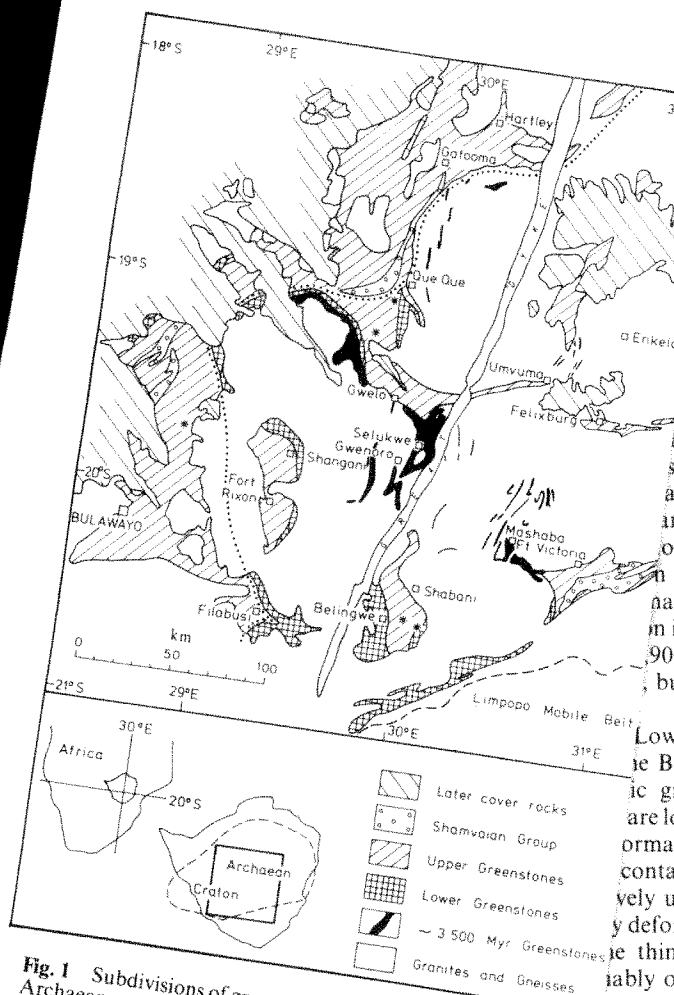


Fig. 1 Subdivisions of greenstone belts in central area of Rhodesian Archaean craton. *, Stromatolites. Dotted line represents approximate division between western and eastern successions of Upper Greenstones.

greenstone belts is the Selukwe area. Here the succession is inverted and forms the lower limb of a large recumbent structure, called the Selukwe nappe¹¹⁻¹⁵. This structure is cut by granite of the Mont d'Or formation dated at ~ 3,400-Myr-old. The rocks of the Selukwe greenstone belt are largely meta-volcanic, ranging from basaltic to peridotitic, but they also include chromitite-bearing ultramafic intrusion. About half-way up the succession is the sedimentary Wanderer formation, the conglomerate of which rests with a marked unconformity on deformed and eroded ultramafic intrusion. Boulders within the conglomerate are particularly significant since they include gneissic tonalite and granodiorite, massive adamellite and pyroxenite¹⁵, indicating the presence of complex sialic crust predating at least part of the ~ 3,500-Myr-old greenstone belts.

The ~ 2,900 Myr terrain

A major ~ 2,900-Myr-old granitic event, which was considered to represent a minimum age for the main greenstone belts, was postulated by Wilson¹⁷ on the basis of conventional K/Ar results¹⁸ from a number of granites and gneisses in the central cratonic area. Subsequent Rb/Sr work disproved this and showed these K/Ar data to be quite unreliable as indicators of age intrusion.

Two more recent Rb/Sr results^{4,5}, however, confirm the presence of ~ 2,900-Myr-old granitic rocks within the cratonic area. These rocks, which form part of the pre-Upper Greenstone basement, are the homogeneous, slightly foliated Mashaba tonalite, which effectively separates the ~ 3,500-Myr-old rocks between Mashaba and Selukwe, and the highly flattened tonalitic Chingezi gneisses¹⁹ on the west side of the Belingwe greenstone belt (Table 1). The Chingezi tonalite, lithologically similar to the Mashaba tonalite, intrudes the Chingezi gneisses and the Lower

Hokonui formation) west of Belingwe. It is so far that it may also be part of the ~ 2,900-Myr-old terrain.

greenstone belts

correlated the main stratigraphic units of the greenstone belt²⁰⁻²³ across the central cratonic area. The belt consists of two sequences, the Lower and Upper Greenstones, separated by an unconformity and by at least one episode of deformation. The Lower Greenstones flank the Upper Greenstones on the western and southeastern sides of the

central (Bend) formation, in the western development of the Lower Greenstones, consists of alternating pillowed basalts, spinifex-textured peridotites and intercalated banded gabbros, capped by a thick conglomerate member which contains an interbedded unit of felsic agglomerate. Boulders in the conglomerate include granitic types. Underlying the Bend formation is the Hokonui formation (Table 1) which consists mainly of felsic flows and pyroclasts. Below the Hokonui formation is a poorly exposed unit of amphibolites, infolded with ~ 2,900-Myr-old Chingezi gneisses. The status of this unit is uncertain, but we have included it with the Lower Greenstones in

the Lower Greenstones on the south-east side of the Belingwe greenstone belt (the Brooklands formation) pass upwards from quartzites, gabbros, granitic grits and conglomerates into high-magnesium lavas which are locally pillowed and alternate with phyllites and banded gabbros. The conglomerates contain tonalite clasts. The contact of the Brooklands formation is not exposed, but its position is indicated by undeformed greenschist facies rocks abut on to completely deformed ~ 3,500-Myr-old gneisses⁵. The thin Manjeri formation forms the base of the unconformably overlying Belingwe Upper Greenstones. It consists of thin developments of clastic shallow water sediments and lime-stones, capped by a persistent horizon of predominantly sulphide-bearing banded iron-formation. On the west side of the belt the Manjeri formation lies on Lower Greenstones; on the east side, it passes into the Brooklands formation to lie with well-exposed Upper Greenstones in conformity on the ~ 3,500-Myr-old Shabani gneisses^{20,21}. Above the Manjeri formation is a thick tholeiitic volcanic sequence of massive and pillowed basalt and metabasalt flows with

Table 1 Rb/Sr whole rock isochron ages and initial ⁸⁷Sr/⁸⁶Sr ratios from the Rhodesian Archaean craton

Rock unit	Age (Myr)*	⁸⁷ Sr/ ⁸⁶ Sr initial
Great 'Dyke' ^{4,7}	2,514 ± 16	0.7026 ± 0.0004
Late Granites—Chilimanzi Suite		
Chilimanzi ^{10,36}	2,620 ± 80	0.7035 ± 0.0080
Zimbabwe ^{10,36}	2,610 ± 60	0.7044 ± 0.0018
Victoria Porphyritic ^{10,36}	2,660 ± 70	0.7025 ± 0.0030
All three on one isochron ³⁶	2,625 ± 25	0.7040 ± 0.0010
Late Granites—Sesombi Suite		
Sesombi ⁶	2,690 ± 140	0.7008 ± 0.0008
Somabula ⁸	2,650 ± 80	0.7012 ± 0.0004
Upper Greenstones		
Maliyami Formation, Que Que area ⁶	2,720 ± 140	0.7010 ± 0.0004
Bulawayo greenstone belt ⁶	2,540 ± 180	0.7015 ± 0.0004
Salisbury greenstone belt ⁷	2,730 ± 60	0.7012 ± 0.0007
Mixed Upper and Lower Greenstones		
Belingwe greenstone belt ⁷	2,760 ± 70	0.7029 ± 0.0002
'Lower Bulawayan', Que Que area ⁶	2,530 ± 280	0.7034 ± 0.0012
Lower Greenstones		
Hokonui Formation, Belingwe ^{4,5}	2,620 ± 120	0.7056 ± 0.0005
Gwenzoro gneisses, south-west of Selukwe ⁶	2,780 ± 60	0.7011 ± 0.0002
Gneisses, Umniati River, Rhodesdale batholith, north-east of Que Que ⁶	2,760 ± 80	0.7015 ± 0.0004
Mashaba tonalite ^{4,5}	2,970 ± 160	0.7013 ± 0.0008
Chingezi Gneisses, Belingwe ⁵	2,884 ± 92	0.7017 ± 0.0005
~ 3,500 Myr terrain		
Mont d'Or formation granite, Selukwe ¹⁶	3,420 ± 120	0.711 ± 0.002
Mushandike granite ^{9,36}	3,520 ± 260	0.7017 ± 0.0030
Gneisses, Shabani area ⁸	3,570 ± 120	0.7000 ± 0.0010
Tokwe Gneisses, Mashaba area ⁶	3,580 ± 400	0.701 ± 0.002

*⁸⁷Rb decay constant $1.39 \times 10^{-11} \text{ yr}^{-1}$. All errors at 2σ level.

Table 2 Simplified western and eastern successions of the Upper Greenstones¹

Western succession	Eastern succession
Calc-alkaline suite of basalt, andesite, dacite flows and pyroclasts; some mafic sills. (? ± 4 km thick)	Not developed
Bimodal volcanic suite: tholeiitic and magnesium-rich basalt and metabasalt pillowed and massive flows, some peridotites; alternating with dacitic flows, tuffs and agglomerates. Grits and conglomerates locally derived from pyroclasts. Sericitic schists. Mafic sills; banded iron-formation phyllites, local limestones in places stromatolitic. (? ± 4 km thick)	Tholeiitic and some magnesium-rich basalt and metabasalt pillowed and massive flows. (? 1–2 km thick)
As for eastern succession	Phyllites, banded iron-formation, local conglomerates and grits; some limestones in places stromatolitic. (Up to 2 km thick)
As for eastern succession	Tholeiitic basalt and metabasalt pillowed and massive flows. Basal development of peridotitic and magnesium-rich basalt flows. Subsidiary sills. Minor pyroclasts. (Up to 7 km thick)
As for eastern succession	Banded iron-formation, local conglomerates and grits, some limestones in places stromatolitic. (Up to 0.25 km thick)

some high-magnesium basalt and subsidiary sills, the whole largely devoid of sediments. The lowermost part of this volcanic sequence is dominated by high-magnesium rocks including pillowed and spinifex-textured peridotite flows and is known as the Reliance formation; the thicker, major part is the Zeederbergs formation^{20,24}. Capping the Zeederbergs formation are the dominantly pelitic shallow-water sedimentary rocks of the Cheshire formation, with locally developed banded iron-formation and limestone.

Figure 1 shows the distribution of the Lower and Upper Greenstones in the central cratonic area¹. Detailed comparisons within the Lower Greenstones are difficult but elements of the Belingwe succession can be recognised. Prominent in all occurrences west of the Great 'Dyke' is a thick unit of Hokonui-type felsic volcanic rocks and derived schists, whereas the linear Mweza greenstone belt²⁵, south-east of Belingwe, contains a sequence possibly transitional between the Bend and Brooklands formations. North-west of Gwelo the Lower Greenstones lie with marked unconformity (refs 1, 26 and D. Edwards personal communication) on presumed ~ 3,500-Myr-old greenstones; elsewhere their lowest preserved sequences are intruded by granitic rocks.

Correlations¹ for the Upper Greenstones in the central cratonic area are, however, clearer. In much of the southwestern part of the area, covered by Fig. 1, the base of the Upper Greenstones is indicated by a thin Manjeri-type marker unit which can be traced for many kilometres and from greenstone belt to greenstone belt. This is succeeded by a thick Zeederbergs-type volcanic pile which is conspicuous in all the major greenstone belts and equates with the basaltic greenstones of Macgregor's Bulawayan System^{27,28}. In several areas the basal part of this succession is a high-magnesium Reliance-type volcanic assemblage. Thereafter, however, above this total volcanic pile the succession in the eastern central cratonic area differs from that in the west¹.

In the east the volcanic pile is capped by Cheshire-type sediments, which are overlain by, and in part interbedded with, a further development of tholeiitic lavas not seen at Belingwe. In the west the thick basaltic pile passes up into repeated bimodal mafic-felsic volcanism on different scales in which tholeiitic and in places high-magnesium spinifex-textured (basaltic and peridotitic) flows alternate with felsic flows and pyroclasts. Sediments derived from the felsic rocks, as well as banded iron-formation and a few limestones, are also a feature of this bimodal succession. Where metamorphosed and deformed, the felsic rocks are sericite schists. Wilson¹ has shown that the western bimodal succession is the time equivalent of, and can be traced laterally into, the eastern pelite-dominant sedimentary unit and its overlying basalts. Suc-

ceeding the bimodal pile is an andesite-dominant, calc-alkaline volcanic assemblage, ranging from basalt to dacite, which was not developed in the east. These two upper western units are confined to a broad zone trending approximately north-north-east across the central cratonic area. In the Que Que and Gatooma areas the bimodal succession includes most of the loosely defined Felsic formation of Harrison^{29,30} and Bliss³¹, whereas the calc-alkaline assemblage embraces the Maliyami formation (Table 1) and the Umniati group respectively of these two authors.

J.F.W.'s¹ correlations across the greenstone belts also allow the rationalisation of the known occurrences of Rhodesian Archean stromatolites which now number five (Fig. 1). All of these occur in the Upper Greenstones. Two occur in limestones of the basal formation below the thick tholeiitic pile; one at Belingwe²⁰, and one at Redcliff south of Que Que (C. A. Castelin, personal communication). Three occur in limestones above the thick tholeiitic pile; one at Belingwe²⁰, one south-east of Redcliff (C. A. Castelin, personal communication) and one, the Huntsman limestone stromatolites of Macgregor³², some 60 km north of Bulawayo.

Table 2 summarises the western and eastern successions of the Upper Greenstones. Figure 2 shows their most likely distribution in the craton as a whole^{1,33,34}.

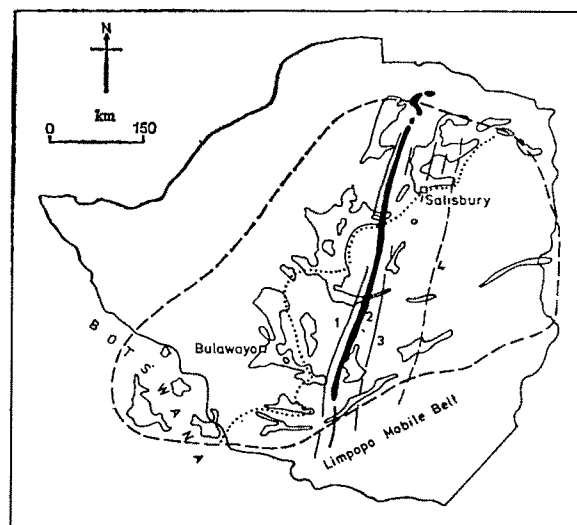
The Shamvaian Group unconformably overlies the Upper Greenstones in the west, east and south-east of the craton¹. The characteristic main rock types are arkoses and sub-greywacke types, rapidly deposited in shallow water and derived from a mixed granitic-volcanic terrain.

Late granites and mafic-ultramafic intrusions

Two suites of late granitic rocks can be recognised post-dating the Upper Greenstones and the Shamvaian Group (Fig. 3). The Sesombi Suite¹ of tonalites and associated granodiorites occurs in the west parallel to the north-north-east trending line of the upper units of the western succession of the Upper Greenstones. The ~ 2,700 Myr ages and the low initial ⁸⁷Sr/⁸⁶Sr ratios of the Sesombi and Somabula tonalites, the two members of the suite so far dated, are indistinguishable from those obtained from the (Maliyami) calc-alkaline volcanic rocks themselves (Table 1). The two intrusions, and by analogy the whole suite, can be regarded as late plutonic expressions of the same magmatic episode which produced the andesites and dacites of the calc-alkaline assemblage. The low initial ratios preclude sources for these volcanics rocks and tonalites in remelting of earlier granite-gneiss terrain^{4,6,35}.

The later ~ 2,600-Myr-old Chilimanzi Suite of intrusions (Table 1) constitute the last major pre-Great 'Dyke' granitic event

Fig. 2 The main greenstone belts of the Rhodesian Archean craton with the Great 'Dyke' and its associated intrusions and major fractures. (1) Umvimeela Dyke; (2) Great 'Dyke'; (3) East Dyke; (4) Popoteke Fault. The dotted line indicates the division between the western and eastern successions of the Upper Greenstones.



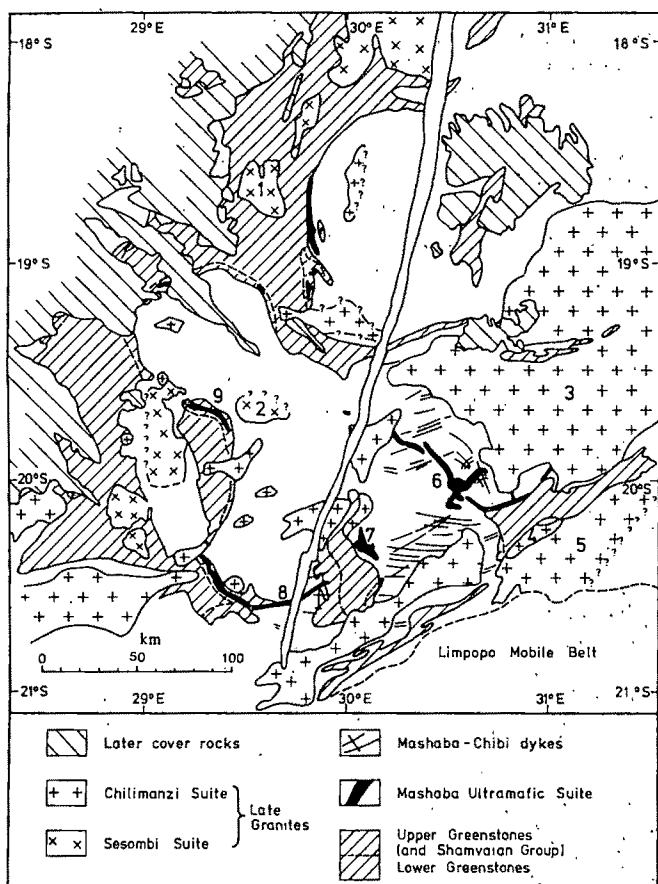


Fig. 3 Distribution of late granites, Mashaba-Chibi dykes and Mashaba Ultramafic Suite in relation to the main greenstone belts in central area of Rhodesia Archaean craton. 1, Sesombi tonalite; 2, Somabula tonalite; 3, Chilimanzi batholith; 4, Victoria Porphyritic granite; 5, Zimbabwe batholith; 6, Mashaba Igneous Complex; 7, Shabani intrusion; 8, Gurumba Tumba-Filabusi intrusion; 9, Shangani intrusion.

in the craton. Petrologically the rocks are dominantly adamellites with local development of granodiorite. The intrusions occur across the craton, but are particularly extensive in the southern and eastern parts. In the southern part of the craton in particular, the intrusions show an east-north-east trend parallel to the Limpopo mobile belt. The strontium isotope data available are compatible with an origin for the Chilimanzi Suite in the remelting of earlier sialic crust^{1,4,36}.

The Mashaba Ultramafic Suite of layered intrusions, together with certain dyke swarms collectively termed the Mashaba-Chibi dykes (Fig. 3), are considered to be broadly contemporaneous with the thick tholeiitic (and high-magnesium) pile which forms the lower part of the Upper Greenstones. None of the intrusions or dykes has yet been dated, but available field evidence places them clearly between the Chilimanzi Suite (~ 2,600 Myr) and the ~ 2,900-Myr-old granitic terrain; they are apparently confined to rocks which pre-date the Upper Greenstones.

The best-documented of the layered intrusions are those of Mashaba and Shabani³⁷⁻⁴⁰, which cut the crustal segment containing the major development of ~ 3,500-Myr-old rocks. The major development of dyke swarms is also within this segment. Some constitute a modified ring and radial pattern and are late phases of the Mashaba Igneous Complex^{37,38}; others are later than the radial swarms and strike east-north-east. The dykes and the Mashaba Igneous Complex also intrude the Mashaba tonalite in this crustal segment.

To the west, dykes and the Gurumba-Tumba Filabusi intrusion cut the Lower Greenstones (Hokonui formation) of the Belingwe belt as well as the Chingezi tonalite and gneisses^{19,23}. North-west of Shangani an undated stock of Sesombi-Suite type truncates the Shangani intrusion¹.

Discussion

The Rhodesian Archaean craton contains granitic rocks intruded during at least three separate events ~ 3,500 Myr, ~ 2,900 Myr and 2,700–2,600 Myr. The ~ 3,500-Myr and the 2,700–2,600-Myr-old events are both associated with greenstone volcanicity and sedimentation. The ~ 2,900-Myr event may well be associated with the Lower Greenstones, if the Chingezi gneisses and Mashaba-type tonalites represent plutonism complementary to the Lower Greenstones felsic volcanicity.

The status of the Gwenoro and Umniati River gneisses is still obscure. They give ages younger than 2,900 Myr ($2,780 \pm 60$ and $2,760 \pm 80$ Myr, Table 1). The Mashaba Ultramafic Suite and the Mashaba-Chibi dykes serve largely to delineate the pre-Upper Greenstones basement. In neither of these two areas of gneisses, however, have representatives of these intrusions or dykes been recognised. Thus these gneisses could be related to an early phase of the ~ 2,700 Myr-old Sesombi Suite. Alternatively they could equally represent a late stage of tonalitic plutonism complementary to the Lower Greenstones, since Stowe's¹¹⁻¹³ mapping supports a pre-Upper Greenstones age for the Gwenoro area gneisses.

The stratigraphic correlations proposed by J.F.W.¹ emphasise that the Upper Greenstones are the remains of a widespread cover sequence which was subsequently deformed to give, very largely, the present configuration of the main greenstone belts. There is now clear evidence of an extensive basement to this Upper Greenstones cover. This basement consisted of the ~ 3,500-Myr-old granite-greenstone terrain, gneisses and tonalites intruded at ~ 2,900 Myr, the Lower Greenstones, and possibly gneisses dated at ~ 2,800 Myr. Remnants can be seen as far east as the (pre-Mushandike granite) gneisses near Fort Victoria, to the Lower Greenstones on the west side of the greenstone belt north of Bulawayo, and east of Que Que (Fig. 1).

The two differing volcanic provinces recorded in the Upper Greenstones would, on any modern analogy, correspond to the constructive and destructive phases of a plate cycle. Perhaps some of the closest modern analogues to the basal sedimentary and volcanic formations are in rifted continental areas, such as Svartenhuk in west Greenland and the Karoo Super Group in southern Africa. Perioditic lavas are apparently restricted to the Archaean, but these younger rifted areas do contain high-magnesium basalts at the base of thick, near sediment-free, piles of basaltic volcanics^{41,42}. What is not clear, however, is whether a tectonic regime responsible for rifting in the Archaean also produced major ocean basins elsewhere, as happened in these Phanerozoic examples.

The present areal distribution of volcanic rocks in the younger parts of the Upper Greenstones, on the other hand, is reminiscent of modern destructive continental margins (Fig. 1); the true western extent of the calc-alkaline suite is obscured by younger rocks, but on present exposures there is a characteristic linear distribution. It is debatable, however, whether this necessitates an origin in a regime analogous to present-day plate tectonics involving subduction. Hawkesworth and O'Nions³⁵, for instance, point out that, in marked contrast to modern plate margin examples, the tholeiites and calc-alkaline rocks of the Upper Greenstones have trace element characteristics which suggest derivation from chemically similar sources. They favour rifting within a continental block for both rock suites which is compatible also with the wide extent of the pre-Upper Greenstones basement as now established. Nevertheless it is intriguing that if a plate tectonic model is adopted, the Upper Greenstones could record both the creation and subsequent destruction of an Archaean ocean basin.

In considering rifting models it is interesting to observe the re-occurrence through time of a near north-northeasterly trend in the Rhodesian Archaean. This is the tectonic trend of the Upper Greenstones, in particular the calc-alkaline suite; it reflects the fundamental grain of the ancient ~ 3,500-Myr-old rocks; and is seen again in the ~ 2,900-Myr-old Mashaba tonalite. More significantly perhaps it is the trend of the Great 'Dyke' fracture system¹. This includes the line of layered mafic-ultramafic

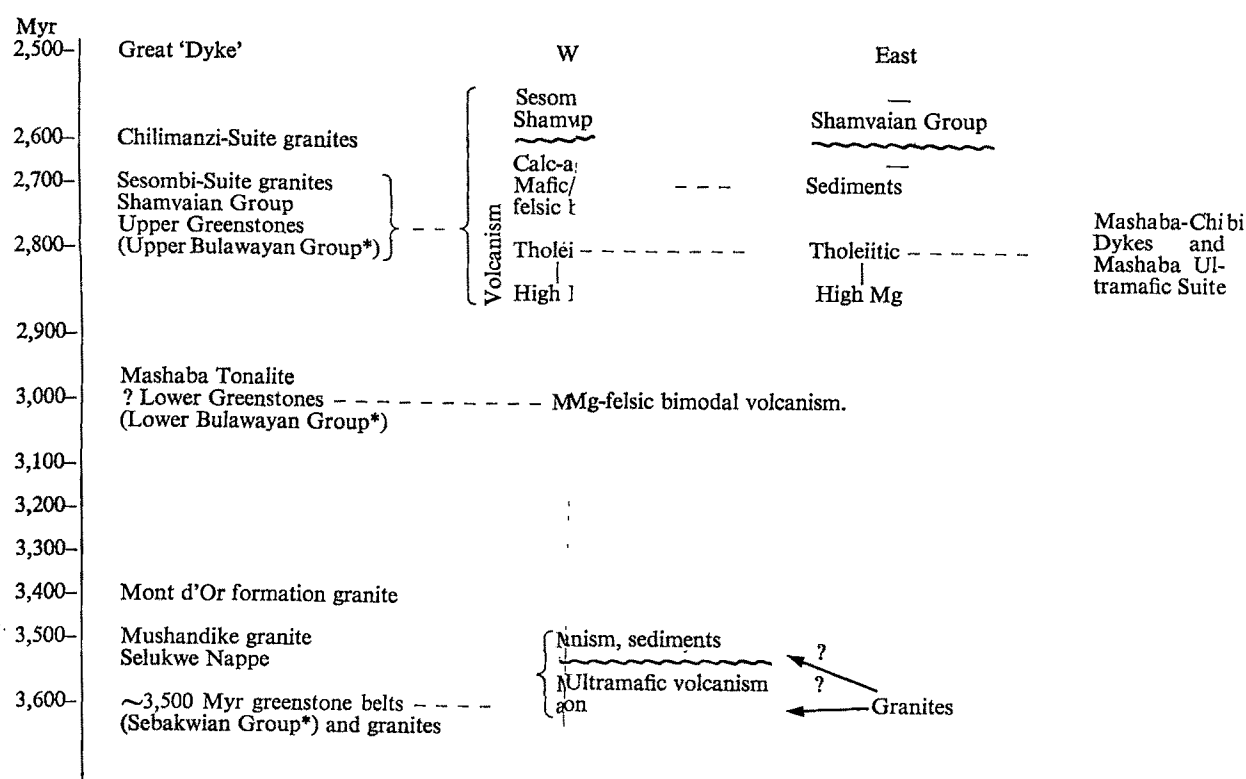


Fig. 4 Summary of major events in the Rhodesian Archaean. *, Nearest equivalent in Rhodesia Geological Survey terminology.

intrusions which constitute the Great 'Dyke' itself⁴³—vimeela and East (true) dykes; and a number of pre-sinistral wrench faults of which the Popoteke fault is the best example (Fig. 2).

Pretorius (in ref. 44) has suggested that the Great 'Dyke' formed in a fracture system which developed in the region of the Rhodesian craton during movements in adjacent belts. Katz⁴⁵ has proposed that the Limpopo mobile belt is a transform zone which, during its initiation, developed greenstone belts on the Rhodesian and Kaapvaal cratons in the system at high angles to it, with subsequent reactivation of the transform zone to produce the rift of the Great 'Dyke'.⁴⁶ We have looked to large intracratonic block movements in deformation in the Limpopo belt and within the Archaean craton.

Accepting a rifting model for the initiation of the Greenstones, and bearing in mind that these ~ 2,700 Myr rocks have not been recognised south of the Limpopo belt, the Kaapvaal craton, it seems reasonable also to accept for intracratonic block movements across what is now the Limpopo mobile belt (and perhaps even also involving a sub-part to the north¹) were accompanied by craton-wide fracturing. An approximately north-north-east trend confined to the Limpopo block, which was effectively the pre-2,700 Myr craton, so that intracratonic block movements, whatever might be their fundamental causes, have been operative over the degrees of intensity from about 2,700 Myr to 2,500 Myr. The Lower Greenstones had an origin in similar earlier events, and some of the Limpopo gneisses are ~ 2,900-Myr. The period is possibly 3,000 Myr to 2,500 Myr. By 2,500 Myr the Rhodesian block, finally stabilised by the wide Chilimanzi-Suite of late granites, fractured sufficiently to form the Great 'Dyke' pattern; and thus, in the Great 'Dyke', the magma of which was some kind of magnesium-rich^{48,49}, we are perhaps seeing a last abortive attempt at an Archaean greenstone belt^{1,50}.

Figure 4 summarises the major events in the development of the Rhodesian Archaean craton.

This paper forms part of the Archaean Crustal Geology of the Department of the University of Rhodesia.

with the Universities of Leeds and Oxford. We thank the Director, Rhodesia Geological Survey, for the use of the unpublished material of A. Martin, D. Edwards, J. D. G. Muirhead, I. D. M. Robertson and M. Hickman, and S. Moorbath, R. K. O'Nions and J. Sutton for their help and interest.

Received 31 August; accepted 4 November 1977.

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Direct comparison of theoretical and experimental melting profiles for RFI Φ X174 DNA

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The determination by Sanger et al. of the complete nucleotide sequence for Φ X174 DNA has made it possible for the first time to compare directly theoretical and experimental DNA melting profiles. The comparison shows that the theory predicts the observed shape of the differential melting curve surprisingly well. Calculation of the denaturation maps allows the peaks on the curve to be correlated with co-operative melting out of concrete regions on the sequence of nucleotides.

HELIX-coil transition or intramolecular melting of the DNA double helix has been studied extensively both experimentally and theoretically for many years (see refs 1-4 for reviews). Until recently the DNA melting curve was thought to reflect rather rough characteristics of base-pair distribution and by the use of it a DNA sequence could be placed only into one of the two categories—quasirandom or block-quasirandom (see refs 1, 2). Recently, however, the viral DNA melting curves have been shown to exhibit a clear-cut fine structure which reflects specific DNA sequences to a much greater extent⁴⁻¹⁰. This fine structure manifests itself most clearly when a derivative of the degree of denaturation with respect to temperature, or differential melting curves, are plotted. Such a plot for a sufficiently short genome consists of a series of sharp peaks with half-width of about 0.3° (see refs 5-10).

There has been considerable progress in the construction of a theory of helix-coil transition in DNA. The theory predicts correctly the main overall characteristics of the process—the melting range width, the number of bases in alternating helical and coiled sections within the melting zone and others. Moreover, the theory has succeeded in predicting the effect of fine structure of the differential melting profiles^{4,5,11}. Sequences in these calculations were simulated with the aid of a generator of random digits because of the lack of data on nucleotide sequence for any DNA: this prevented the direct comparison between the theory and experiment. The situation has changed now that Sanger *et al.*¹² have published the complete nucleotide sequence for Φ X174 DNA. In the present work we are using these data to compare directly, for the first time, theoretical and experimental differential melting profiles.

Recent advances in theory

A model that is used in the theory of helix-coil transition is rather simple (see refs 1-4 for reviews). Each base pair is assumed to belong to one of the two states—'helical' or 'coil-like'. Opening of a base pair at the end of a helix needs free energy ΔF_{AT} for an AT pair and ΔF_{GC} for a GC pair. Formation of a new unwound region inside the helix needs an additional energy, F_s , that may be considered as the energy of base-pair stacking. In addition to these ordinary features of the well known Ising model, the DNA model includes so-called loop-weighting factors. These statistical weights appear because unwound regions in DNA have the shape of a closed polymer chain.

Comparison of theoretical and experimental melting profiles

Closed circular double-stranded replicative form (RFI) of Φ X174 DNA is not of interest for our purposes because both theoretical

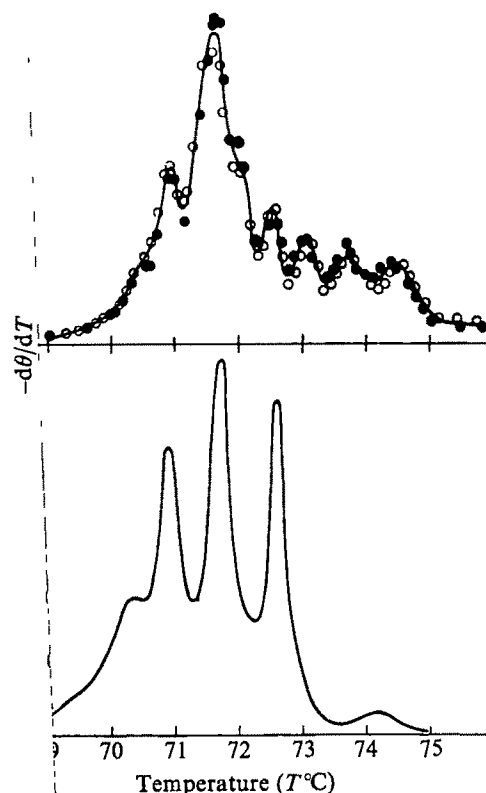


Fig. 1 (a) Experimental and (b) theoretical differential melting profiles of RFI Φ X174 DNA in 0.1SSC. The theoretical curve was calculated using the sequence of nucleotides determined by Sanger *et al.*¹² and the theory of helix-coil transition in DNA using the standard parameters^{1,2,4,5,18}: $\sigma = 5 \times 10^{-5}$; $\alpha = 1.5$; $U_{AT} = -1$, $T_{AT} = 52.5^\circ\text{C}$; $T_{GC} - T_{AT} = 42.4^\circ\text{C}$. Replicative form (RFI) of Φ X174 DNA was obtained from the cells of *Escherichia coli* infected by phage Φ X174, the multiplicity of infection 10, with subsequent treatment by chloramphenicol. The Φ X174 of wild type and *E. coli* C cells were gifts of Drs E. S. and L. Patrushev. The chromosomal DNA was removed by centrifugation (40,000g, 1 h) and the supernatant was suspended in 50% isopropanol. The separation of RFI DNA mixture of RFI and the linear DNA molecules was done by centrifugation in density gradient of CsCl with addition of ethidium bromide (200 $\mu\text{g}/\text{ml}^{-1}$)²². Ethidium bromide was removed by isopropanol extraction and subsequent dialysis against SSC buffer (0.15M NaCl, 0.015M Na citrate). The sample contained about 90% of RFI DNA and 10% of R. Conversion of RFI DNA into RFII DNA was done using nuclease¹⁹ in 0.2M CH_3COONa , pH 4.6 in the presence of ZnSO_4 at 37°C ; sufficient enzyme was added to half of the DNA (5 $\mu\text{g}/\text{ml}^{-1}$) in 10 min. The reaction was followed by appearance of RFI band in the course of Agarose gel electrophoresis. Incubation for 30 min led to the complete disappearance of RFI DNA. At the same time about 10% of the molecules were converted into linear form. Melting of DNA was followed by appearance of RFI band in the course of Agarose gel electrophoresis. Incubation for 30 min led to the complete disappearance of RFI band. Differential melting profiles were obtained by graphical differentiation and automatically²³. The profiles obtained by the two methods and in many independent measurements were very close. The filled and open circles on the experimental curve (a) are two examples of such measurements. The long plateau at high temperatures represents contamination, probably due to host DNA.

predictions and experimental observations led to the conclusion that such DNA molecules should have broad but structureless differential melting profiles⁵. So theoretical calculations and experimental investigations should be conducted in RF II DNA having a single-stranded break (nick).

We have converted RF I DNA into RF II using S1 endonuclease. This enzyme was shown by Kato *et al.*¹⁹ to introduce the nicks into RF I Φ X174 DNA by a random way. The theoretical profile was obtained by averaging over ten calculated curves differing one from another by a position of nick. The shape of the theoretical differential melting profile depends on the position of the nick but this dependence is not strong—the peaks are shifted by not more than by 0.1–0.2 °C.

Theoretical and experimental differential melting profiles thus obtained are given in Fig. 1. In general, the curves are remarkably alike. Indeed, positions of peaks at 70.4, 70.9, 71.0 and 72.6 °C coincide for theoretical and experimental profiles with as high precision as of about 0.1 °C. But the amplitudes of the peak at 72.6 °C are very different for both curves and, beginning with this peak, the curves differ from one another considerably. We shall discuss the possible causes of these discrepancies (below).

Correlation of theoretical denaturation maps with melting profile

In addition to the melting profile, the theory permits the calculation of a denaturation map, that is, the dependence of the probability of a base pair to be open on its position within a nucleotide sequence.

Figure 2 shows theoretical denaturation maps calculated for RF II Φ X174 DNA in different temperatures. Values of the temperature are indicated to the right. These maps being compared with the differential melting profile presented in Fig. 1 enable us to correlate the peaks on the profile with co-operative melting of concrete regions on the nucleotide sequence published by Sanger *et al.*¹².

Until recently a rigorous and effective algorithm for calculating a melting curve for DNA with an arbitrary sequence of base pairs has been lacking. Such an algorithm has now been formulated as the result of the following sequence of events.

Lacombe and Simhar¹³ developed the unique probability formalism allowing the calculation of equilibrium characteristics for a linear heterogeneous system. This formalism was different from, but equivalent to the ordinary formalism based on the partition function method. Poland¹⁴ showed that this formalism made it possible to treat rigorously the DNA model. By the method of Poland, and the previously proposed method of Wartell and Montroll³ based on ordinary formalism, were not sufficiently effective—the computer time required was proportional to N^2 where N is the number of base pairs in the chain. An approximate method¹⁵ was proposed as early as 1969 for which the computer time is proportional to N . This method has been used extensively in this laboratory^{1,2,5}. Recently Lukashin *et al.*¹⁶ have compared the results of calculations by this method with the results obtained by the exact methods^{3,14} and concluded that the approximation¹⁵ gives results which are in accord with results of exact methods. These findings stimulated Fixman and Freire¹⁷ to introduce an idea that formed the basis of the method¹⁵ into formalism^{13,14}.

Fixman and Freire¹⁷ succeeded in formulating an exact algorithm for which computer time is proportional to the number of base pairs in DNA. So now, at last, theoreticians have an exact and highly effective method¹⁷ for treating the DNA model. This method is used here to calculate differential melting profiles and denaturation maps for RF II Φ X174 DNA.

Parameters of the theory

The theory of helix-coil transition makes it possible to compute a melting curve and a denaturation map for DNA if a sequence of base pairs and values of parameters of the theory are given. The theoretical parameters are as follows (see refs 1–4): the melting enthalpy for an AT pair, U_{AT} ; the melting temperature of

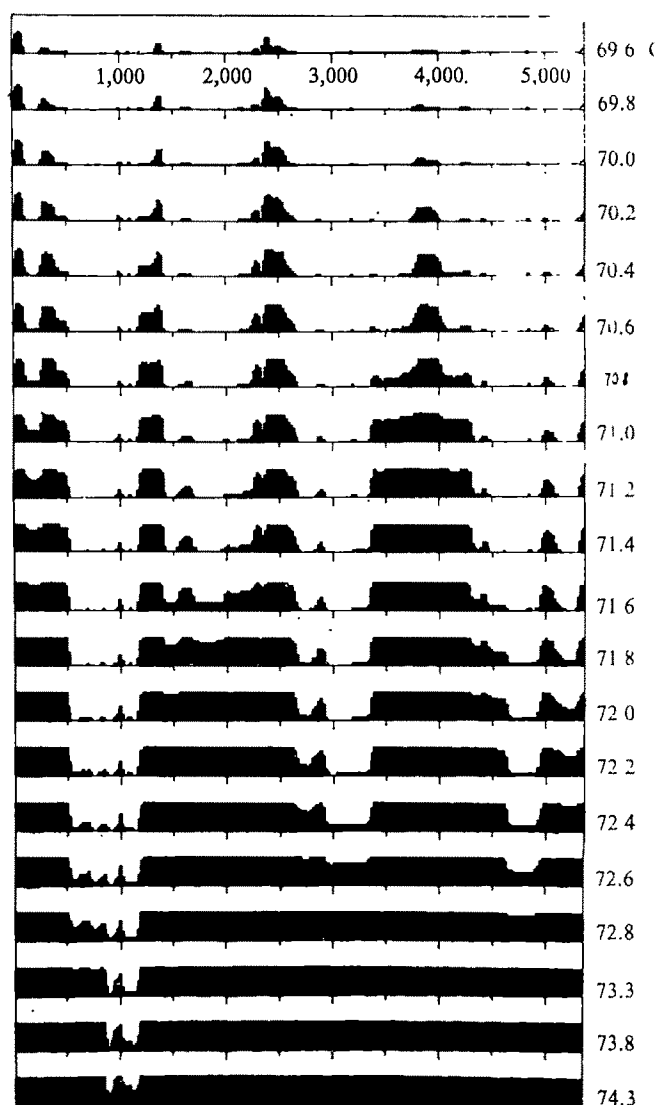


Fig. 2 Theoretical denaturation maps for RF II Φ X174 DNA calculated with the same set of parameters as indicated in the legend for Fig. 1. As for the melting profile, each map was obtained by averaging over ten maps differing from one another by the position of the nick. The sequence is numbered as in Fig. 1 of ref. 12. An ordinate presents the probability of a base pair to be open averaged over 25 subsequent base pairs.

poly AT; T_{AT} ; the difference $T_{GC} - T_{AT}$; the co-operativity factor, σ ($\sigma = \exp(-F_s/RT)$); and loop-weighting exponent α .

The value U_{AT} has been determined from calorimetric and other data and it is equal to 8.0 kcal/mol (see, for example, ref. 4). The values of T_{AT} and $T_{AT} - T_{GC}$ depend on ionic conditions and are determined from a series of experiments with DNA having different GC content. For conditions used in the present work (0.1SSC), $T_{AT} = 52.5$ °C and $T_{GC} - T_{AT} = 42.4$ °C, see ref. 18.

The value of σ is chosen as a rule to be equal to 5×10^{-5} and $\alpha = 1.5$ (see refs 1,2,4,5). A considerable ambiguity exists in this choice of values—the value of $\alpha = 1.8$ is more justifiable from the theoretical point of view than the value of 1.5 and that of σ is not known more accurately than to within an order of magnitude. Fortunately, as appropriate calculations have shown, the differential melting profile, at least in our case, is practically unaffected by the change of these two parameters within the limits of their possible variations. In general, our calculations show that the shape of differential melting curve is highly sensitive to the sequence of base pairs and to the values of parameters U_{AT} and $T_{GC} - T_{AT}$ but insensitive to the particular values of the rest of parameters.

The regions melting at the lowest temperatures are too short to give distinct peaks on the differential melting profile. Nevertheless the shoulder at 69.3 °C may be recognised on the theoretical profile originating from the melting out of the region, which is situated, as one may conclude from Fig. 2, between bases with order numbers 25 and 100. Inspection of the corresponding piece of the nucleotide sequence¹² shows that this region is highly enriched (by about 20%) by AT pairs as compared with the sequence as a whole.

The first distinct peak at 70.4 °C appears mainly due to a co-operative melting of the region situated between 3.80 and 4.00 kilobases. Then the two neighbouring regions situated at 3.35–3.80 and 4.00–4.30 kilobases are melting out simultaneously giving the large peak at 70.9 °C. The main peak at 71.7 °C originates from a co-operative melting of the very large region containing about 850 base pairs situated between 1.40 and 2.25 kilobases. Simultaneously the region between 4.30 and 4.65 kilobases melts. The melting of this region that may explain, at least in part, the peak at 72.0 °C on the experimental profile. Indeed, such a peak, but with much lower magnitude than the experimental one, appeared on theoretical profiles for some positions of the nick.

The large peak at 72.6 °C on the theoretical profile corresponds to a simultaneous melting of two independent regions at 2.90–3.35 and 4.65–4.95 kilobases. Finally the last small peak on the theoretical profile originates from the melting of the region positioned between 1.00 and 1.15 kilobases. The small area which remains completely helical up to the highest temperature is situated, as it can be seen from Fig. 2, at 0.87–0.90 kilobases. Inspection of the nucleotide sequence¹² in this site shows that starting from 872-th nucleotide the sequence of 35 bases highly enriched by GC pairs follows. It contains only 8 AT pairs and it has higher GC content, compared with the mean value, by more than 25%. This GC cluster remains completely helical up to about 73.5 °C.

It should be remembered in this connection that the theory of helix-coil transition in DNA used here is valid under the assumption that complete dissociation of complementary strands does not take place. This assumption is valid until a single helical region exists, that is, up to about 73.5 °C, for our particular case, and it fails for higher temperatures. So, at best, accordance between the theory and experimental results may be expected only for the temperatures lower than 73.5 °C. We can conclude that the only serious difference between the theoretical and experimental melting profiles is the too large a magnitude of the peak at 72.6 °C on the theoretical curve. Indeed the melting of the region situated between 0.50 and 0.85 kilobases (see Fig. 2) should give a peak at 72.8 °C, which may be considered as corresponding to the peak at 73.0 °C on the experimental curve. On the theoretical curve (see Fig. 1) it is masked by a powerful peak at 72.6 °C.

It should be emphasised that in general it is absolutely impossible to predict, just by inspecting a sequence of nucleotides, what particular region would melt out co-operatively to result in a peak on the differential melting profile. Only for two extreme regions, highly enriched by AT pairs and highly enriched by GC pairs, does the simple explanation of their behaviour, given above, exist, but these sequences are too short to give a distinct peak on the profile. For the rest of the sequence it is only the theory of helix-coil transition in DNA that makes it possible to indicate the regions which are melting out co-operatively and to correlate them with peaks on the differential melting curve.

Note also that the melting temperature of a region depends not only on its GC content but also on its length and boundary conditions. For example, the region at 0.50–0.85 kilobases contains about 47% GC, the region at 4.65–4.95 kilobases contains about 50% GC but the former melts at a slightly higher temperature than the latter in contrast to the expectation based only on their GC content. The causes are clear—the region at 0.50–0.85 kilobases had a melted area only to its left before melting, but the region 4.65–4.95 kilobases had the melted areas from both sides. Thus considerable errors may follow from an attempt to correlate

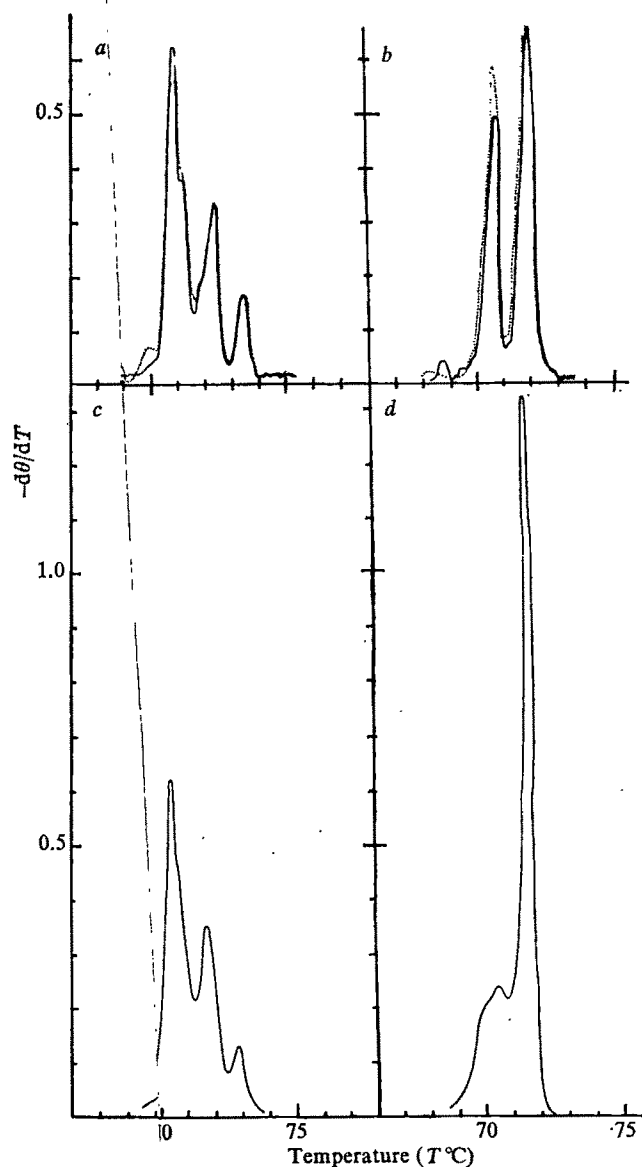


Fig. 3 Theoretical (c, d) and experimental (a, b) differential melting profiles for reaction fragments Y_1 (a, c) and Y_2 (b, d) of $\Phi X174$ DNA in a 0.1 M aqueous solution. Experimental profiles are taken from Wada *et al.*²⁷. Solid and dotted curves correspond to different experiments and demonstrate reproducibility. Theoretical curves were calculated using the parameters listed in Fig. 1 legend.

directly the position of a peak on a differential melting curve with GC content of a corresponding region.

Possible causes of discrepancy

The above discussion shows that the only important discrepancy between the theoretical and experimental melting profiles is the difference of magnitudes of the peak at 72.6 °C for both curves. Special calculations excluded an ambiguity in the values of parameters σ and α as a possible cause of this difference. Two causes that may be responsible for it are discussed below.

First the real sequence of nucleotides in the DNA studied by us experimentally may differ from the sequence published by Sanger *et al.*¹². The phages used by Sanger *et al.*¹² and by us are of different origin but they probably differ from one another by only a few point mutations. Possibly of more importance, however, as Sanger *et al.*¹² emphasised, occasional errors might be introduced into the sequence during the laborious experiments involved in the determination. The regions where such errors may occur are shown in Fig. 1 of their paper¹². In the four positions the bases are not indicated at all. We have filled these gaps in the following way: 1,421, A; 1,422, A; 1,423, C and 1,657, C. These few uncertainties cannot affect the results presented above. But our results may be influenced by the fact that a very long region exists starting at 2.95 and extending to 3.68 kilobases where one mistake

per 50 residues, and in some places even greater level of uncertainty, is possible. Indeed, one of the regions contributing to the peak at 72.6 °C on the theoretical curve in Fig. 1, the region 2.90–3.35 kilobases, is situated almost completely within this most uncertain area. Note that most of the theoretical profile presented in Fig. 1 cannot be changed because it is formed by the melting of sequences fully confirmed.

Second, the effect of heterogeneity of stacking interactions between base pairs neglected in the theory, may influence the results. This effect is large for RNA helices according to available data²⁴. But, in the case of DNA this effect is shown to be very small by direct experimentation with DNA²⁵ as well as by the theoretical analysis²⁶ of experimental data on melting of synthetic polynucleotides with known sequences. The above results show the theory to be successful in precisely predicting positions of a series of the peaks on the differential melting curve for Φ X174 DNA; this constitutes evidence for a neglected role of the effect of heterogeneity of stacking interaction. Nevertheless, we cannot yet exclude completely a possibility that in some special situations this effect may give an appreciable contribution.

It is difficult to draw an unambiguous conclusion from this work because of the possible errors in the published sequence. Nevertheless the results obtained strongly suggest that the theory can efficiently predict the shape of a differential melting curve in detail. The model which forms the basis of the theory is thus appropriate to real DNA.

We thank Dr A. V. Lukashin for help, Drs Y. S. Lazurkin and V. I. Ivanov for discussions, Mrs V. N. Syomushkina for technical assistance and Dr M. Fixman for sending a preprint of his paper.

After completion of this study we became acquainted with the very interesting data of Wada *et al.*²⁷. These authors measured

the differential melting curves for two restriction fragments of Φ X174 DNA: Y₁ consisting of 2,745 base pairs (position 3,360–729) and Y₂ consisting of 1,690 base pairs (position 1,104–2,793). We have calculated the theoretical melting profiles for these fragments which are presented in Fig. 3 along with the experimental profiles taken from ref. 27.

Received 22 July; accepted 20 October 1977.

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Comparison of amino acid sequence of troponin I from different striated muscles

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The sequence of troponin I from fast and slow skeletal and cardiac muscle shows strong homology in the region which binds to actin and is responsible for inhibition of the actomyosin ATPase. More differences are found in the N-terminal region which binds to troponin C.

CONTRACTILE activity in vertebrate striated muscle is regulated by the level of free Ca²⁺ in the sarcoplasm by means of the molecular switch formed by the troponin complex and tropomyosin. Troponin is composed of three components, troponin I, which inhibits the actomyosin ATPase, troponin C, which binds Ca²⁺ and troponin T, which binds to tropomyosin and locates the complex at a repeat of 385 Å along the thin filament^{1,2}. Troponin I will inhibit the actomyosin ATPase on its own but this inhibition is greatly enhanced in the presence of tropomyosin³. The inhibition is neutralised by troponin C and the restoration of full Ca²⁺ sensitivity requires troponin T^{4,5}. Each component of the thin filament can be shown to interact with two others to bring about this regulation and it has been shown that troponin I interacts with both actin⁶ and troponin C⁷, this latter interaction being Ca²⁺ sensitive. The primary structures of all the major thin filament proteins from rabbit fast skeletal muscle are known^{8–12} but while the pattern of regulation in the other major types of vertebrate striated muscle seems similar, the troponin components have been shown to differ in different muscle types^{13–15} and this may well reflect modifications in

regulatory activity. We have determined the amino acid sequence of four troponin I proteins from different sources in order to investigate the molecular basis of the interaction with the other thin filament proteins, troponin C and actin, and also to elucidate the differences between proteins obtained from different types of striated muscle. The sequences of three of the proteins chosen, fast¹¹ and slow¹⁶ skeletal and cardiac¹⁷ troponin I from the rabbit, have been published and we report here the sequence of troponin I from chicken fast skeletal muscle. A detailed description of the determination of this sequence will be published elsewhere.

Primary structure determination

The amino acid sequences of the four proteins are shown in Fig. 1. The four sequences have been aligned to give maximum homology and it will be seen that a number of deletions have to be introduced into each sequence. The numbering of the residues is that for the cardiac protein, which includes two deletions at positions 181 and 182. One correction must be made to the previously published sequence of the rabbit fast muscle protein¹¹. The sequence in the region 139–143 was previously thought to be Leu-Arg-Arg-Arg-Val but has now been shown to be Leu-Arg-Arg-Val. The corresponding sequence in the rabbit cardiac protein is Leu-Arg-Leu-Arg-Val, this was noted previously¹⁷ to be in some doubt but efforts to clarify this sequence were unsuccessful. A blocked N-terminus was found in each protein.

	10	20	30
C.F.			X-Ser
R.F.			Ac-Gly-Asp-Glu-Glu-
R.S.			NH-Pro Val-
R.C.	X-Ala-Asp-Glu-Ser-Arg-Asp-Ala-Ala-Gly-Glu-Ala-Arg-Pro-Ala-Pro-Ala-Val-Arg-Arg-Ser-Asp-Arg-Ala-Tyr-Ala-Thr-Glu-Pro-His-Ala-		
	40	50	60
C.F.	Lys-Arg Ala	Ala Leu Val Ile	Ala
R.F.	Lys-Arg-Asn-Arg-Ala-Ile-Thr-ALA — ARG Arg-Gln-His-LEU-LYS-Ser-Val-MET-LEU-Gln-Ile-ALA-Ala-Thr-Glu-Leu-Glu-Lys-Glu-Glu-		
R.S.	Glu Lys-Ser-Lys Ser Lys-Leu — Leu Ala-Lys Lys-Glu-Cys-Gln-Gln-Glu-His		
R.C.	Ser-Lys-Lys-Lys Ser Ser Lys-Leu-Gln Thr-Leu Lys-Gln Arg Ala-		
	70	80	90
C.F.	Ala-Ala-Lys Val		Gln Leu
R.F.	Gly-Arg-Arg-Glu-Ala-GLU-LYS-Gln-Asn-Tyr-LEU-Ala-Glu-His-Cys-Pro-Pro-LEU-Ser-Leu-Pro-GLY — Ser-Met-Ala-Glu-Val-GLN-Glu-		
R.S.	Ala — Val-Arg Arg-Ile Ala Gln-Thr-Arg Leu Leu-Ser-Ala-Leu Asp-		
R.C.	Glu-Glu Arg-Gly Gly-Arg-Ala Ser-Thr-Arg Gln Glu Ala Leu-Gly-Phe Leu Asp		
	100	110	120
C.F.	Lys Ser-Val-Asp Arg Thr Val Leu Thr-Asn		
R.F.	LEU-CYS-Lys-Gln-LEU-HIS-ALA-Lys-Ile-Asp-Ala-Ala-Glu-GLU-GLU-Lys TYR-ASP-Met-GLU-Ile-LYS-Val-Gln-Lys-Ser-Ser-Lys-GLU-Leu		
R.S.	Arg Val-Glu-Val-Val-Asp Arg Ile Ala Cys-Leu-His-Asn-Thr-Arg Ile		
R.C.	Arg Arg-Val Lys-Val-Asp Arg Val Ala Thr Asn-Ile-Thr Ile		
	130	140	150
C.F.	Leu-Ser		
R.F.	Glu-ASP-Met-Asn-Gln-LYS-Leu-Phe-ASP-LEU-ARG-GLY-LYS-PHE-LYS-ARG-PRO-Pro-LEU-ARG — ARG-VAL-ARG-Met-SER-ALA-ASP-ALA-MET		
R.S.	Lys Leu-Lys-Leu Val-Leu Val		
R.C.	Ala Leu-Thr Ile Thr Leu Ile		
	160	170	180
C.F.	Arg Asn		
R.F.	Leu-Lys-ALA-LEU-LEU-GLY-Ser-Lys-His-LYS-Val-Cys-Met-ASP-LEU-ARG-ALA-Asn-LEU-LYS-Gln-VAL-LYS-LYS-GLU -ASP-THR-GLU-LYS-GLU		
R.S.	Arg Ser Ser		
R.C.	Met-Gln Thr-Arg-Ala Glu-Thr-Leu His		
	190	200	210
C.F.	Lys-Asp-Leu Ala-Gly		
R.F.	— — — Arg-Asp-VAL-GLY-ASP-TRP-ARG-LYS-ASN-Ile-Glu-Glu-Lys-SER-GLY-MET-GLU-GLY-ARG-LYS-LYS-Met-PHE-Glu-Ser — Glu		
R.S.	Arg-Pro-Val — Glu Val Ala-Met Asp-Ala-Ala-Lys		
R.C.	Asn Glu Asp-Leu-Leu Lys Gly- COOH		
	215		
C.F.	- COOH		
R.F.	Ser- COOH		
R.S.	Pro-Thr-Ser-Gln- COOH		

Fig. 1 Alignment of the amino acid sequences of troponin I from chicken fast muscle (C.F.), rabbit fast muscle (R.F.), rabbit slow muscle (R.S.) and rabbit cardiac muscle (R.C.). The complete sequence of rabbit fast muscle troponin I is presented, gaps in the other sequences indicate identity with this sequence. Residues which are identical in all four sequences are shown in capitals. Solid lines represent deletions. X indicates an unknown blocking group.

In the rabbit fast muscle protein this has been shown to be an acetyl group¹¹ and this is likely to be the case for the other proteins as all other myofibrillar proteins so far studied have been shown to be N-acetylated. One curious feature of the rabbit slow muscle protein is that although some of the molecules have a blocked N-terminus, most have proline with a free imino group and we believe this to be the first example of a myofibrillar protein with a free N-terminus. Heterogeneity was also found at the C-terminal

end of the molecule where a proportion of the molecules lacked the terminal Ser-Gln dipeptide. The origin of these different forms of slow muscle troponin I is not clear, but as the protein was isolated by affinity chromatography after extraction of muscle with 9 M urea it is unlikely that they are an artefact of preparation. Two other myofibrillar proteins, the A1 and the 'phosphorylatable' light chains of myosin^{18,19}, have been shown to have a blocked N-terminal proline, but in both cases blocking seemed to be complete.

Comparison of sequences

The four complete troponin I sequences have been compared by calculating the percentage difference between pairs of proteins (Fig. 2). As the proteins differ slightly in length, extra residues at either the N- or C-terminus were ignored and deletions were treated as an extra amino acid. The proteins were also divided arbitrarily between residues 119 and 120 and the N- and C-terminal parts were compared. The matrices (Fig. 2) show that the fast muscle proteins from both chicken and rabbit are rather closely related and have only half the number of differences found between any two of the three rabbit proteins. In the case of the three rabbit proteins the number of differences between any two of them are approximately the same. When the N- and C-terminal halves are compared this general pattern is still maintained but mutations have accumulated in the N-terminal half at approximately twice the rate observed in the C-terminal half. There is some indication that the N-terminal halves of the rabbit slow and cardiac troponin I are more similar than either is to the rabbit fast troponin I and also that the C-terminal halves are correspondingly more different, but it is doubtful if this is significant.

The only other component of the troponin complex for which comparative sequence data are available are troponin C. The sequences of three fast skeletal muscle troponin C components have been published, from rabbit⁸, chicken²⁰ and human muscle²¹ and also the sequence of one cardiac troponin C from beef²². The difference between the rabbit and chicken proteins is 11% while there is only one difference between the rabbit and human proteins. All three fast muscle proteins differ from the cardiac protein by between 30% and 35%. This suggests that troponin C is a somewhat more conservative protein than troponin I.

In a comparison of the sequences the most striking feature is the extra 26 residues which the cardiac protein has at the N-terminal end which are not shared by the proteins from either fast or slow muscle. Apart from the N-terminal region the greatest differences are found in the region of residues 181–184 where one, two and three deletions have to be introduced into the rabbit slow, cardiac and fast muscle sequences respectively to fit them to the chicken fast muscle sequence. There is no discernable pattern in the sequence in this region and this is made more curious by the fact that the sequence on either side of this region is invariant. The sequence of the rabbit slow muscle protein includes a proline at residue 182 and this region may represent a bend in the polypeptide chain where mutations can be tolerated without affecting the overall three-dimensional structure.

Predictions of the secondary structure have been made^{23,24} and give values of about 45% α -helix and 10% β -sheet in rabbit skeletal troponin I whereas values determined from circular dichroic and optical rotary dispersion methods give values of 29% and 20% for α - and β -structure respectively²⁵, which are somewhat at variance with the predictions. There are two regions where concentrations of proline residues are found—in the regions of residues 76 and 137. The predicted structures emphasise that these are likely to be associated with bends in the structure, dividing the molecule into three roughly equal sections. These considerations, together with the highly polar nature of the proteins suggest that they exist as fairly open structures. In each case the charged amino acids account for some 40% of the residues and each protein possesses a net positive charge, this being 8 and 9 respectively in the case of rabbit and chicken fast muscle troponin I and 14 and 18 in the case of rabbit cardiac and slow muscle troponin I. The higher charge on the slow muscle protein is a consequence not only of an increased number of lysine and arginine residues but also of a decrease in the number of

aspartic and glutamic acid residues, there is not however any marked difference in the charge distribution in each molecule.

Studies on the phosphorylation of the rabbit fast and cardiac muscle troponin I show that a number of sites can be phosphorylated using phosphorylase kinase and a cyclic AMP-dependent protein kinase^{26–28}. The position of these sites is shown in Fig. 3.

The major phosphorylation sites in the rabbit fast muscle protein are at residues 37 and 146 (threonine 11 and serine 117 in the fast muscle troponin I sequence¹¹). Phosphorylation at both these positions is blocked in the presence of troponin C and it is doubtful if phosphorylation at these sites is of any physiological significance although they might play a part in the initial assembly of the troponin complex. In each of the proteins there is either a serine or a threonine present at positions 37 and 146 and the sequence around these residues shows considerable homology, it is possible that each of these positions may be phosphorylated in appropriate conditions, but with the exception of serine 146 (ref. 28) in cardiac troponin I this has not yet been demonstrated. The major phosphorylation site in cardiac troponin I is at serine 20^{28,30}, which is in the extra N-terminal part of the sequence. This residue is rapidly phosphorylated by cardiac cyclic AMP-dependent protein kinase and since it has been shown by Solaro *et al.*³⁰ to be phosphorylated when the perfused heart is stimulated with adrenaline, it is tempting to speculate that the part of the sequence which is unique to the cardiac protein is of functional importance in the regulation of cardiac muscle contraction.

Fig. 2 Difference matrices for the amino acid sequences of troponin I components. The numbers shown are % differences between sequences, ignoring extra terminal residues and counting deletions as an extra amino acid. C.F., chicken fast muscle; R.F., rabbit fast muscle; R.S., rabbit slow muscle; R.C., rabbit cardiac muscle. *a*, Comparison of whole proteins; *b*, comparison of sequences up to residue 119; *c*, comparison of sequences from residue 120 to the C-terminus.

	CF	RF	RS	RC
CF	0	18	39	42
PF	18	0	40	41
RS	39	40	0	40
RC	42	41	40	0
<i>a</i>				
	CF	RF	RS	RC
CF	0	27	55	56
RF	27	0	55	54
RS	55	55	0	48
RC	56	54	48	0
<i>b</i>				
	CF	RF	RS	RC
CF	0	10	22	28
RF	10	0	25	27
RS	22	25	0	33
RC	28	27	33	0
<i>c</i>				

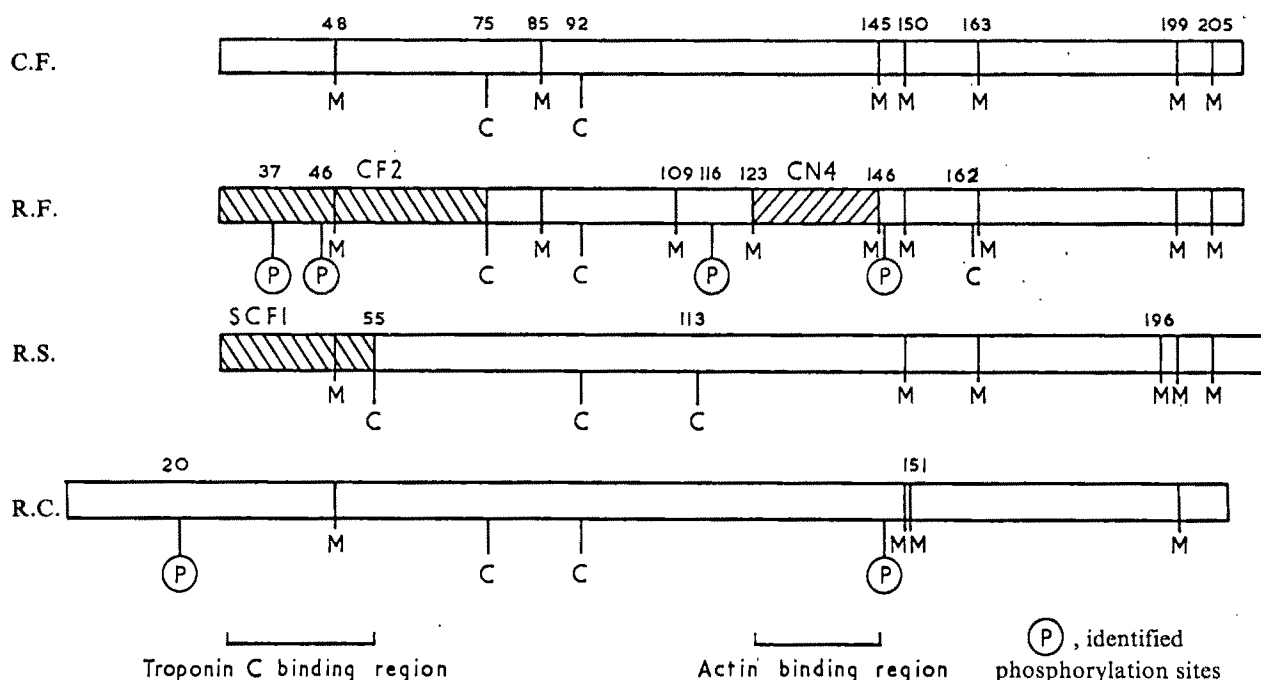


Fig. 3 Schematic representation of troponin I proteins from chicken fast (C.F.), rabbit fast (R.F.), rabbit slow (R.S.) and rabbit cardiac (R.C.) muscle. C, cysteine residues; M, methionine residues. The numbering system is that for the cardiac protein as shown in Fig. 1. In the R.F., R.S. and R.C. proteins only unique identified phosphorylation sites, C and M residues are numbered. The shaded areas indicate the fragments which interact with troponin C (CF2 and SCF1) and actin (CN4) as discussed in the text.

Biological activity of troponin I peptides

Two regions of the rabbit fast muscle troponin I molecule have been implicated in interactions with other components of the thin filament by Syska *et al.*³¹. The cyanogen bromide fragment CN4 (residues 124–145) has been shown to inhibit the actomyosin ATPase, this inhibition being enhanced by tropomyosin and reaching up to 70% of the inhibition obtained with whole troponin I and tropomyosin. This fragment has also been shown by affinity chromatography to bind to actin and also, to some extent, to troponin C. The troponin C binding region has been located at the N terminus of the molecule, partly by the inhibition of threonine-37 phosphorylation²⁹ and more particularly by the demonstration of the binding of the cysteine cleavage fragment CF2 (residues 27–74) to troponin C, both by affinity chromatography and gel electrophoresis³¹.

A consideration of the methionine positions in each of the proteins (Fig. 3), shows that methionine 123 is unique to rabbit fast muscle troponin I. It has probably arisen fairly late in the evolution of fast muscle troponin I and is therefore unlikely to occur in proteins from other sources. It is thus not possible to prepare analogous fragments from the other proteins in order to compare their inhibitory activity. Inspection of the sequence in this region (Fig. 1) shows, however, that it is one of the most strongly conserved in all of the four proteins. With the exception of residue 124, where all the proteins differ, the replacements in general involve strongly conservative hydrophobic residues. There is also a Thr/Pro replacement at position 138 in the cardiac protein and a possible extra leucine at position 141, which has been discussed above. It seems unlikely that any of these changes would affect the inhibitory activity significantly and if there are real differences in this their cause must be sought in other parts of the troponin I structure. This constancy of sequence at the actin binding site is perhaps not surprising in view of the very conservative nature of actin itself.

It is difficult to define the troponin C binding site as precisely as that for actin binding as appropriate fragments are not available. The cyanogen bromide fragment CN5

(residues 27–48) from the rabbit fast muscle protein shows some troponin C binding activity but is not nearly as effective as CF2. It is probable that the binding site spans methionine 48 but may not extend as far as cysteine 75. The rabbit slow muscle protein has an extra cysteine at residue 55 and the fragment produced by cleavage at this residue has been shown to bind to a troponin C–Sepharose column¹⁶, but owing to incomplete cleavage the amount of fragment obtained was small and no quantitative estimate of binding could be obtained. On the basis of homology the most likely binding site is between residues 36 and 52 but even in this region there is considerable variation.

As noted above, the inhibitory peptide (residues 124–145) has some binding affinity for troponin C. Weeks and Perry³² have shown that two of the large cyanogen bromide peptides from troponin C will neutralise the inhibition of the actomyosin ATPase by troponin I and will also inhibit phosphorylation at residues 37 and 146. It is not clear, however, if these observations indicate a second specific binding site for troponin C on the troponin I molecule, in the same position as the actin binding site, or whether they are due to strong ionic interactions between highly acidic peptides and a highly basic region in troponin I.

It is clear from the comparison of the sequences that the genes coding for the three types of striated muscle troponin I diverged at much the same time in evolutionary terms and well before the divergence of birds and mammals.

The overall rate of evolution has been significantly greater than that for troponin C and this must be a reflection of the differing constraints placed on the tertiary structures. Troponin C has four Ca^{2+} binding sites and also interacts with two other components of the thin filament. When one of the Ca^{2+} binding sites is lost, as in the cardiac protein²², the sequence in this region is found to vary considerably. Troponin I has only two protein binding sites and thus a higher rate of mutation is to be expected. But, as shown above, the N- and C-terminal parts of the molecule have changed at rather different rates—the C-terminal half having much the same rate of evolution as troponin C whereas the N-terminal half has changed at nearly twice this rate.

The constancy of the C-terminal parts is explicable in terms of binding to the strongly conserved actin molecule and this would suggest that the mechanism for the inhibition of the actomyosin ATPase is the same for each of the three types of striated muscle troponin I. The much greater variability in the N-terminal part of troponin I and in particular in the troponin C binding region is somewhat surprising in view of the conservative nature of troponin C itself and this may reflect subtleties in the interaction of troponin I with troponin C which may only be appreciated from an understanding of the three-dimensional structure of the troponin complex.

We thank Professor S. V. Perry and Dr A. J. G. Moir for discussions and the MRC for financial support.

Received 2 August; accepted 20 October 1977.

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letters to nature

Discovery of an X-ray QSO

WE report here the discovery of an X-ray emitting QSO, the first to be initially identified from X-ray observations. Previously, the only QSO known to be an X-ray source was 3C273 (refs 1 and 2). The new QSO has been found within a 40'' error circle established by the SAS-3 X-ray Observatory. The SAS-3 error circle lies within the Ariel V error box (~0.2 square degrees) for the source 2A 2251–179 (ref. 3). Following the convention for optical QSOs, we have designated the optical object MR2251–178. The X-ray luminosity (2–11 keV) of this object, presently $\sim 5 \times 10^{44}$ erg s⁻¹, has been as large as $\sim 1.6 \times 10^{46}$ erg s⁻¹ (in 1975) and exceeds the optical luminosity by a factor of ~10. Among known compact X-ray sources, only 3C273 has a greater luminosity. Also, in radio observations at 4,885 MHz with the NRAO Very Large Array (VLA), we have discovered a point-like radio source coincident with MR2251–178.

A 12° × 12° field near 2A2251–179 was observed with the rotating modulation collimator (RMC) system (ref. 4) on the SAS-3 X-ray Observatory from 11.1 August 1977 to 13.5 August 1977. Data from 36 orbits (effective integration time of 9.7×10^4 s) have been analysed.

Detection of a source within the error box for 2A2251–179 was achieved in each of the two independent RMC detectors. The statistical significance of the detection was 6.2σ in the 2!3 FWHM detector (2–11 keV) and 5.7σ in the 4!5 FWHM detector (2–11 keV). In Table 1 the results of these two independent detections are given, including spectral data for the

two energy channels in each of the two detectors. While the spectral shape cannot be uniquely determined, the data are consistent with a power law having a photon number index of 1.5 ± 0.5 (assuming $E_0 \gtrsim 2$ keV; ref. 5). The flux is $2.5 \pm 0.4 \times 10^{-11}$ erg cm s⁻¹ (2–11 keV). If we combine the two independent position determinations according to the technique described by Doxsey *et al.*⁴ we obtain the following position for the X-ray source (in subsequent catalogues, the X-ray source will be designated 2S2251–178).

$$\alpha(1950) = 22 \text{ h } 51 \text{ min } 25.4 \text{ s}, \quad \delta(1950) = -17^\circ 50' 40'' \\ \text{error circle radius (90\% confidence): } 40''$$

In Fig. 1, we show the Ariel V error box for 2A2251–179 (0.187 square degrees in area; ref. 3) and the refined SAS-3 error circle reported here. Both are superimposed on a Palomar Observatory Sky Survey (POSS) blue plate. Figure 1 inset shows only the SAS-3 error circle, but with a scale factor twenty times larger. Within the SAS-3 error circle, there are only 2 conspicuous objects ($m_v \lesssim 20$) visible on the POSS plates. They are denoted '1' and '2' on the inset. Object 1 possibly is a compact elliptical galaxy, whereas Object 2 is starlike. There is no marked difference between the red and blue POSS images of either object. Coordinates for these two objects were measured on POSS glass plates at Kitt Peak National Observatory (KPNO) by S. Kleinmann. Plates from the Tololo–Michigan Objective Prism Survey (previously taken with the 61 cm Curtis Schmidt telescope at Cerro Tololo; refs 6 and 7) were then examined. On these plates, the spectrum of Object 1 is obscured in part by that of Object 2. In the most remarkable spectrum of Object 2, bright emission lines from the following species are evident: Hβ, [OII] λ3727, [NeIII] 3869, Hγ, and probably [NeV] λ3426 and HeII λ4686. Using 4 lines in the 3,500–5,350-Å interval, we determined a redshift of $z = 0.0680 \pm 0.003$. This implies a distance of 410 Mpc, assuming $H_0 = 50$ km s⁻¹ Mpc⁻¹. Based on the diameter of its POSS image, the apparent magnitude of Object 2 is $B \sim +16$.

Table 1. X-ray observations of MR2251–178 (2A2251–179) by SAS-3

Collimator f.w.h.m.	Energy band (keV)	Counting rate (s ⁻¹)
2!3	2–6	0.45 ± 0.08
	6–11	0.14 ± 0.05
4!5	2–6	0.30 ± 0.06
	6–11	0.13 ± 0.05

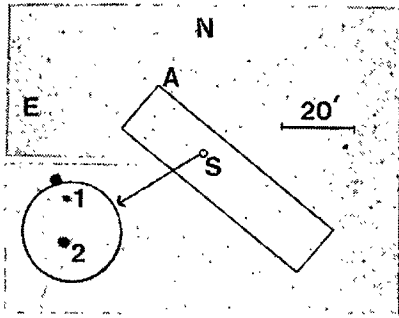


Fig. 1 The QSO MR2251-178 and the X-ray source 2A2251-179. 'A' and 'S' denote the Ariel V error box and the SAS-3 error circle, respectively. The inset shows the SAS-3 error circle enlarged by a factor of 20. Object 2 is MR2251-178. Photographs are taken from the Palomar Sky Survey Plates National Geographic Society).

The B luminosity of the object is then $\sim 5 \times 10^{43}$ erg s $^{-1}$, while the X-ray luminosity is $\sim 5 \times 10^{44}$ erg s $^{-1}$ (2-11 keV). The position of the optical object (designated MR2251-178) is

$$\alpha(1950) = 22 \text{ h } 51 \text{ min } 25.89 \pm 0.03 \text{ s}, \\ \delta(1950) = -17^\circ 50' 54''.2 \pm 0''.5$$

The coordinates of the centroid of Object 1 were also measured at KPNO. They are

$$\alpha(1950) = 22 \text{ h } 51 \text{ min } 25.82 \pm 0.03 \text{ s}, \\ \delta(1950) = -17^\circ 50' 15.3'' \pm 0.5''$$

A search for radio emission from MR2251-178 was carried out at a frequency of 4,885 MHz using 6 antennas of the NRAO Very Large Array (VLA) in New Mexico. Operating with 50 MHz bandwidth and system temperatures of roughly 60 K, the six 25-m antennas form an aperture synthesis system capable of easily detecting 1 mJy sources in a 5' field in 1 h. With the six antennas arranged in a linear array, 7.7 km in length, the resolution of the system is < 1 arc s. Aperture synthesis maps of the field containing MR2251-178 made with 1.8 and 2.4 h of data on 4 and 6 October, 1977, independently show the presence of a dominant 3.2 ± 0.3 mJy point source at a position of

$$\alpha(1950) = 22 \text{ h } 51 \text{ min } 25.90 \pm 0.04 \text{ s}, \\ \delta(1950) = -17^\circ 50' 53.5'' \pm 1''$$

No variability was noted between the two dates and the source was < 1 arc s in extent.

In the field containing MR2251-178, there is also an extended radio source of very low surface brightness at

$$\alpha(1950) = 22 \text{ h } 51 \text{ min } 25.8 \pm 0.8 \text{ s}, \\ \delta(1950) = -17^\circ 50' 20'' \pm 10''$$

This source has a size of the order of 30'' and contains at least 10 mJy of radio flux at 4,885 MHz. Because of the elongation of the synthesised beam, it is not possible at the present time to discuss the detailed structure of this extended source; however, considering its position and size, it is very likely associated with Object 1 in Fig. 1.

Based on the positional coincidence (~ 40 arc s), we regard the identification of the X-ray source 2A2251-179 with the optical object MR2251-178 as virtually certain. The association of the VLA radio point source with MR2251-178 is certain (~ 1 arc s positional coincidence). The classification of MR2251-178 as a QSO is based upon its stellar appearance on POSS plates, its redshift, and its high luminosity. It is possible that it could be an extreme Seyfert galaxy. This, however, would require that its luminosity be greater than

that of any known X-ray-emitting Seyfert galaxy (refs 8 and 9). 2A2251-179 is reported to vary by a factor of ~ 10 in the 2A catalogue (ref. 3). At the maximum brightness reported, the 2-11 keV luminosity would be $\sim 1.6 \times 10^{46}$ erg s $^{-1}$. This exceeds the maximum value reported for the Seyfert galaxy of highest reported X-ray luminosity, 3C390.3 (refs 10 and 11).

Among the known compact X-ray sources, only 3C273 has a maximum luminosity greater than MR2251-178 (ref. 10). The ratio of X-ray to optical emission we have observed for MR2251-178 is $\sim 10:1$ compared to a ratio of $\sim 1:1$ in 3C273 and typical X-ray Seyferts (ref. 10). Thus, judging from available data, the energy budget of MR2251-178 is dominated by its X-ray emission. Accurate assessments of its bolometric luminosity must await infrared and high energy (> 10 keV) X-ray measurements.

The redshift of MR2251-178 would place it among the closer QSOs. In all, there are only 5 (out of 633) QSOs in the list of Burbidge, Crown, and Smith (ref. 12) which have smaller redshifts. At the redshift distance of MR2251-178, the projected separation between it and Object 1 is ~ 75 kpc.

The presence of the extended radio source near Object 1 may suggest a possible link to MR2251-178. A redshift measurement of Object 1 would clarify this possibility.

The optical and radio properties of MR2251-178 are such that it is unlikely that it would have been detected in pre-Tololo surveys. In the optical, it would likely have been passed over as an uninteresting field star, while in the radio, it is too faint to have been detected in any standard survey to date. Thus, there could be many such sources. Even if their number density were as great as that of Seyfert galaxies ($\sim 0.5\%$ of all galaxies; ref. 13), they could have been overlooked in optical searches, since the objects would be ~ 2.5 mag fainter for a given X-ray luminosity than are the Type 1 Seyfert galaxies studied by Ariel V (ref. 8), of which the optically faintest was $m_v \sim 14.5$. In view of the hard spectrum of MR2251-178 in the X-ray region (comparable in index to the diffuse X-ray background; ref. 14), the general population of such objects could make a substantial contribution to the X-ray background.

We acknowledge support from the staffs of the Center for Space Research at MIT and the Goddard Space Flight Center of NASA. We thank H. Bradt, C. Canizares, S. Kleinmann, R. Kelley, W. Lewin, J. McClintock, and B. Rossi of MIT, and B. Cooke, M. Elvis and J. Pye of the Ariel V Group, University of Leicester for helpful discussions and assistance. The National Radio Astronomy Observatory is operated by Associated Universities, Inc. under contract with the US NSF. This work was supported in part by NASA under contract NAS5-11450.

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Received 12 October; accepted 24 October 1977.

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Observation of X-ray eclipses from LMC X-4

THERE are six known X-ray sources in the Magellanic Clouds: five in the Large Cloud and one in the Small Cloud^{1,2}. Until recently, only SMC X-1 was known to be in a binary system³. Chevalier and Ilovaisky⁴ have now shown that the optical emission of the suggested counterpart of LMC X-4 (ref. 5) exhibits ellipsoidal light variations with an inferred binary period of 1.408 d. Previous X-ray observations have indicated that LMC X-4 is highly variable on time scales from minutes to months^{2,6–8}. We report here the detection of several eclipses of LMC X-4, which confirms it as the second known extragalactic X-ray binary. We also discuss briefly the inferred value of the mass of the compact X-ray star.

LMC X-4 was observed on four occasions with the X-ray detectors on SAS-3. The first observation⁶ was made with the Rotation Modulation Collimator system (RMC) during 20.5–26.4 February 1976 (UT). During the interval 15.8–17.6 October 1976 (UT), LMC X-4 was scanned twice per satellite orbit

with the Right Slat detector system^{9,10}. The Y-axis detectors^{9,10} were then pointed continuously at LMC X-4 during the intervals 22.2–23.2 and 23.99–24.32 May 1977 (UT). During the first of these pointed observations, SAS-3 was operated with a special telemetry mode whereby the data from the 6–12 keV channel of the Horizontal Tube system^{9,10} were recorded with a temporal resolution of 8 ms (ref. 11). For the second pointed observation, as well as the scanning and RMC observations, the normal 0.83 s time resolution of the satellite was used.

LMC X-4 was extremely weak compared to the non-X-ray background during most of our observations. We were, therefore, unable to obtain accurate intensities for the source. Instead, we assigned a qualitative description to the observed intensities as

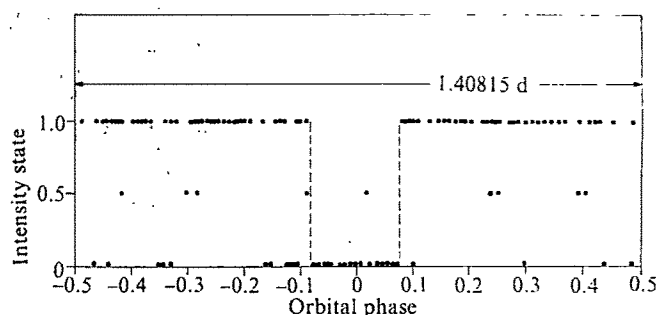


Fig. 1 Orbital X-ray light curve for LMC X-4. The 'digitised' X-ray intensities for LMC X-4, given in Table 1, are folded modulo the trial orbital period 1.40815 d. The apparent eclipse of the X-ray source has a duration of 0.23 ± 0.02 d and is centred at JD 2443068.01 ± 0.02 .

Table 1 Observed intensities for LMC X-4

Time*	Intensity† state	Time	Intensity state	Time	Intensity state
28.952	1	33.747	0	268.708	0
29.410	1	33.811	1/2	268.823	1
29.477	1	33.878	1	268.839	1
29.607	1	33.942	1	268.888	1
29.871	1	34.009	0	268.954	1
29.937	0	34.073	0	269.020	1
30.132	1	34.140	0	269.035	1
30.198	1	34.207	0	269.085	1
30.265	1	34.271	0	269.151	1
30.331	1	34.338	0	269.167	1
30.395	1	267.375	1	269.216	0
30.463	1	267.398	1	269.232	1
30.527	1	267.440	1/2	269.282	0
30.593	1	267.538	0	269.560	1
30.791	1	267.561	0	269.576	1
31.053	1	267.635	1	269.695	1
31.120	1	267.658	1	269.761	1
31.184	1	267.725	1	269.826	1
31.251	0	267.835	1	269.871	1
31.315	0	267.857	0	269.937	1
31.382	0	267.900	1/2	270.002	1/2
31.514	0	267.923	0	270.021	1
31.654	1	268.034	0	270.068	1
31.711	1	268.051	1/2	485.773	1
31.775	1	268.100	0	485.839	1
31.842	1	268.117	0	485.970	1
31.906	1	268.165	1	486.101	1
31.973	1/2	268.182	1	486.167	1
32.105	1	268.231	1	486.298	0
32.169	0	268.248	1	486.364	0
32.236	1	268.297	1	486.429	0
32.301	1	268.314	1	486.495	1
32.367	1	268.362	1/2	486.626	1
32.433	1/2	268.379	1/2	486.757	1
32.497	1	268.428	1	487.552	1
32.760	0	268.445	0	487.618	0
32.827	0	268.493	1	487.683	0
32.891	0	268.511	1	487.749	0
33.549	1	268.576	1	487.814	1
33.616	0	268.625	1		
33.680	1	268.642	0		

*JD – 2442800.0

†See text for definition.

follows: 1 when the intensity was at least 3σ above the background, 0 when it was less than 1σ above the background and 1/2 when we could not determine definitively whether the source was 'on' or not. We then compiled a list of these intensity states, one for each scan during the scanning observation and one for every satellite orbit during the other observations. The results are presented in Table 1.

It is evident from Table 1 that LMC X-4 was 'off' for ≥ 5 h on six separate occasions. This behaviour is similar to the eclipses observed in other eclipsing X-ray binaries^{1,2}. Furthermore, the last two 'off' states occurred very close to the times when the unseen secondary was predicted to be behind the primary⁴. Unfortunately, the orbital period determined from the optical data is not sufficiently accurate to determine whether the earlier X-ray 'off' states occurred at a predicted eclipse time also.

The X-ray 'intensities' from all four observations were folded modulo trial binary orbital periods between 1.404 d and 1.412 d in steps of 0.0001 d. The period range is chosen to be within two standard deviations of the optical period⁴. Figure 1 displays the 'light' curve for the trial orbital period of 1.40815 d. An eclipse is clearly discernible with a width of 0.23 ± 0.02 d. (It is difficult to assign a formal confidence to the errors quoted in this paragraph: however, we regard these errors as conservative limits.) When the data were folded at periods different from 1.40815 d by more than 0.00020 d, the resulting 'eclipses' were significantly shorter and eventually disappeared. We conclude that we have observed an X-ray eclipsing phenomenon with the above binary period, and with an eclipse centre at JD 2443068.01 ± 0.02 . This confirms the proposed optical identification of LMC X-4.

A search for X-ray pulsations in the data taken with 8 ms and 0.83 s time resolution was carried out. No significant peaks in the Fourier power density spectrum were observed. From these studies we place an upper limit to pulsing of $\sim 25\%$ of the steady flux with pulse periods ranging from ~ 16 ms to 400 s. We note that if LMC X-4 had a pulsation fraction and pulse shape similar to that of SMC X-1 (refs 13, 14) it would have been only marginally detectable as a pulsar.

From the velocity amplitude of the He(II) emission lines in the LMC X-4 binary system ($475 \pm 25 \text{ km s}^{-1}$; ref. 4), we estimate a lower limit (2σ) to the mass function:

$$f(M) = \frac{m_{\text{opt}}^3 \sin^3 i}{(m_{\text{opt}} + m_x)^2} = \frac{4\pi^2 (a_x \sin i)^3}{G P_{\text{orb}}^2} \gtrsim 11.2 M_{\odot}$$

where m_{opt} and m_x are the masses of the companion star and X-ray star, respectively, i is the orbital inclination angle, $a_x \sin i$ the projected semi-major axis of the orbit of the X-ray star, and P_{orb} the orbital period. To establish the limit on the mass function we have assumed that the He(II) emission arises from a region between the two stars⁴. We further define the mass ratio of the two stars as $q = m_x/m_{\text{opt}}$.

Figure 2 is a plot of contours of constant m_x in the q/i plane for a mass function $f(M) = 11.2 M_{\odot}$. The upper limit⁴ on the velocity of the optical companion [$\sim 79 \text{ km s}^{-1}$ (2σ)] implies that the mass ratio, q , is smaller than ~ 0.17 . Chevalier and Ilovaisky⁴ argued that i must be greater than $\sim 70^\circ$, because of the large amplitude of the ellipsoidal light variations. The measured X-ray eclipse half-angle, θ_e , is between 27° and 31° . If we assume that the optical companion fills at least 90% of its Roche lobe, then the above limits on q , i , and θ_e restrict m_x to be greater than $\sim 1.1 M_{\odot}$. (For a more complete discussion of this method of mass estimation, see refs 15, 16.) As the value of $f(M)$ we adopted is only a lower limit, we cannot provide a meaningful upper limit to m_x . The shaded region of Fig. 2 shows the allowable range of values of m_x for the various constraints imposed. If $f(M)$ is larger than $11.2 M_{\odot}$, the contours of constant m_x will shift to the lower right of the q/i plane, thereby increasing the lower limit on m_x . We note that if the data point with 'intensity' = $1/2$, which occurs during

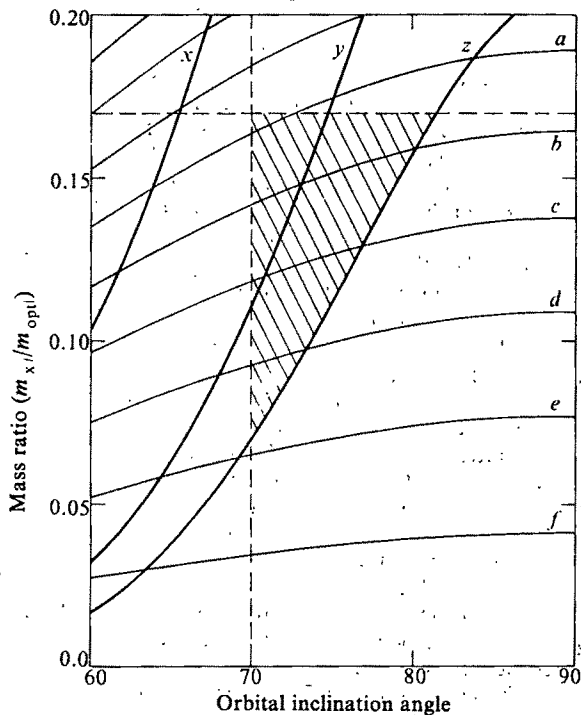


Fig. 2 Constraints on the mass, m_x , of the X-ray star in LMC X-4. The light solid curves are contours of constant m_x in the mass ratio/orbital inclination angle (q/i) plane (see text), for a mass function $f(M) = 11.2 M_{\odot}$: a-f are for $m_x = 3, 2.5, 2, 1.5, 1$ and $0.5 M_{\odot}$, respectively. The heavy solid curves are constraints imposed by the assumption of various orbital geometries and eclipse angles; θ_e (see ref. 14): x, y and z represent $(1.0 R_T, \theta_e 27^\circ)$, $(1.0 R_L, \theta_e 29^\circ)$, and $(0.9 R_L, \theta_e 31^\circ)$, respectively, $1.0 R_T$, $1.0 R_L$, and $0.9 R_L$ denotes cases where the optical companion star fills 100% or 90% of its critical tidal or Roche lobe, respectively. The other constraints on q and i are discussed in the text. The hatched region denotes the acceptable range of values of m_x for $f(M) = 11.2 M_{\odot}$. For larger values of $f(M)$ the contours of m_x shift down and to the right in the plot; all other features remain fixed.

the eclipse (Fig. 1), actually represents an 'on' state, the eclipse half-angle would then be reduced to $\sim 20^\circ$. This also has the effect of increasing the lower limit on m_x .

Since the completion of this work, we have become aware of a similar study¹⁷ by the X-ray group at the Mullard Space Science Laboratory. The values reported for the X-ray eclipse ($0.206 \pm 0.008 \text{ d}$) and the orbital period ($1.413 \pm 0.007 \text{ d}$) are in good agreement with the corresponding values quoted in the present work, though of differing precisions.

The authors wish to thank the SAS-3 group for their assistance during this study and, in particular, G. Jernigan and P. Joss for helpful discussions and P. Hertz for preparing the figures. S.R. acknowledges support from the Alfred P. Sloan Foundation. This work was supported in part by NASA.

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Discovery of eclipsing nature of LMC X-4

THE Sanduleak-Philip optical candidate for LMC X-4 (ref. 1) has been reported by Chevalier and Ilovaisky² to be part of a 1.4-d binary system. Six days of data taken from an extended Ariel V observation of the LMC are shown in Fig. 1 and X-ray eclipses 5 h long can be clearly discerned every 1.4 d. The predicted optical phase zero times are, within the uncertainties, in good agreement with the observed mid-eclipse times, thus confirming the identification and making this the first X-ray eclipsing binary system to be found in the LMC.

The 3.75° FWHM field of view of the proportional counter spectrometer (PCS) was centred on LMC X-4 but also contained LMC X-1, LMC X-3 (ref. 3) and AO538-66 (refs 4, 5). The three latter sources plus contributions from the particle and X-ray backgrounds are responsible for the residual flux seen during LMC X-4 eclipses. The gaps in the data are caused by the satellite turning off during its night time, regions of high charged particle background and lost data. In spite of this, both eclipse ingress and egress were seen on two occasions and from these an orbital period of $1.413 \pm 0.007 \text{ d}$ was obtained (phase zero occurring on JD 2443342.614), in good agreement with the optical value of $1.408 \pm 0.002 \text{ d}$ ². Combining the X-ray and optical epochs (obtained 4 months apart) gives an improved period of $1.40838 \pm 0.00027 \text{ d}$.

Figure 2 shows one of the eclipses plotted with a time resolution of 64 s. Entry and exit occur on a timescale of minutes with an eclipse duration on the two occasions where it could be measured of $0.208 \pm 0.008 \text{ d}$ ($\theta_e = 26.2 \pm 1.1^\circ$). This may be combined with the available optical data to derive a mass for the secondary, but this is dependent both on the inclination of the system and the

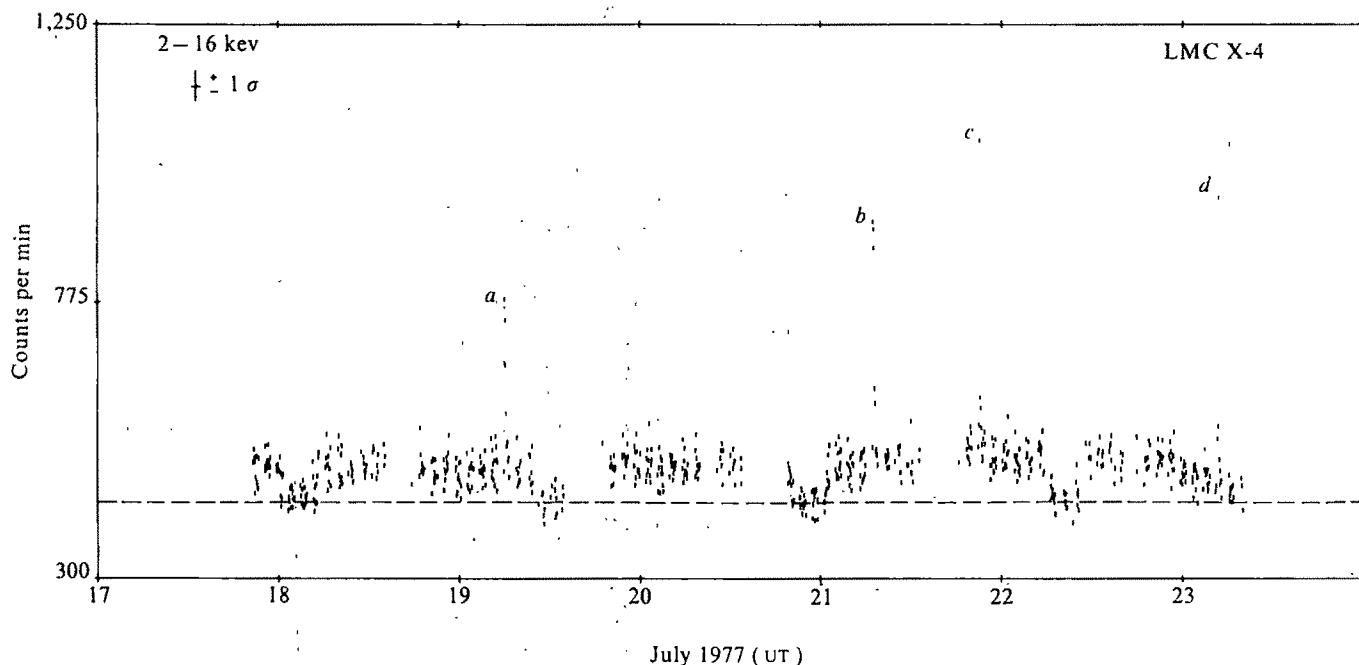


Fig. 1 The flux seen by the PCS integrated in 8 min time bins. The decline in flux during the last few hours can be attributed to the pointing position moving away from LMC X-4, LMC X-1 and LMC X-3.

assumed radius of the primary star. At present, these two parameters are not sufficiently constrained to improve significantly the estimate of $1-3 M_{\odot}$ already derived by Chevalier and Ilovaisky².

The X-ray data were unsuccessfully searched for evidence of periodicities between ~ 1 s and 40 min with an upper limit of

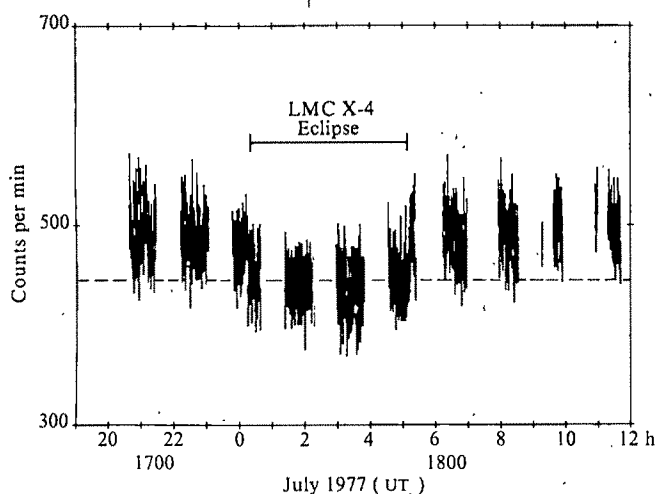


Fig. 2 One eclipse from LMC X-4 plotted with the finest time resolution available (64 s).

$\sim 20\%$ the mean flux. The overall peak luminosity of LMC X-4 was $\sim 3.4 \times 10^{-10} \text{ erg cm}^{-2} \text{ s}^{-1}$ in the 1.7 to 17.0 keV band which represents a total source output of $1 \times 10^{38} \text{ erg s}^{-1}$. X-ray eclipses were only seen between 15 and 23 July, although the observations lasted from 29 June to 23 July and from 3 to 7 August. Six days of data obtained from 30 June onwards were folded modulo the orbital period and a two sigma upper limit to any residual flux above background (as defined during the predicted eclipse) of $1.8 \times 10^{-11} \text{ erg cm}^{-2} \text{ s}^{-1}$ was found. This represents an upper limit to the source luminosity of $5 \times 10^{36} \text{ erg s}^{-1}$.

Four flares each lasting between 10 and 20 min were seen and they are shown in Fig. 3 plotted with the shortest time resolution available of 64 s. These 'outbursts' could have originated from any source in the field of view of the PCS. The PCS, however, is coaligned with a rotation modulation collimator which can obtain a source intensity averaged over one satellite orbit (typically ~ 50 min out of every 100) and combining two of the orbits when the flares occurred indicated a factor two increase in the intensity from LMC X-4 (G. Carpenter, personal communication). This suggests that the outbursts did originate from LMC X-4 and that the peak represents a source luminosity of $9 \times 10^{38} \text{ erg s}^{-1}$. We note that Epstein *et al.*⁶ have reported 20-s

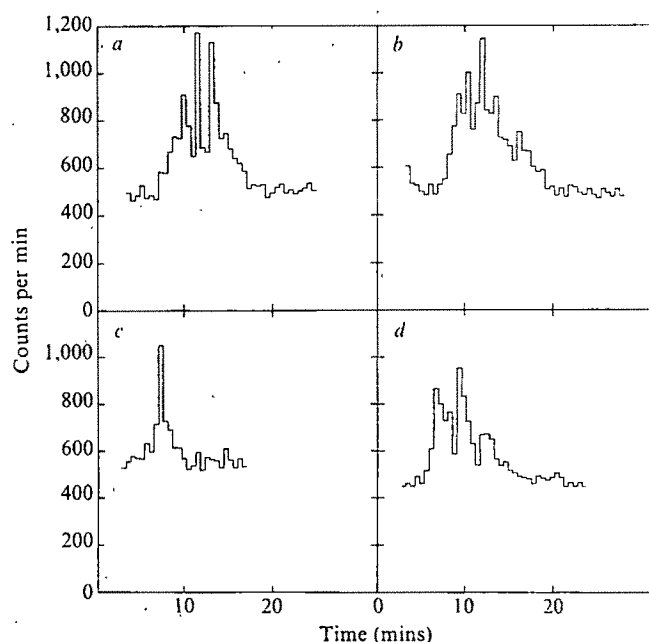


Fig. 3 Four outbursts plotted with 64 s time resolution. The times (a, b, c, d) at which they occurred are indicated in Fig. 1. The 1σ uncertainties are the square root of the count rate.

events of comparable luminosity from the vicinity of LMC X-4 and these may be related to the four outbursts reported here.

I thank G. F. Carpenter of Birmingham University and P. J. N. Davison for useful discussions.

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Received 10 October; accepted 3 November 1977.

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A search for rapid optical periodicities from Cyg X-1

ACCORDING to the most widely accepted model of the X-ray source Cyg X-1, the X rays are produced in a gas disk surrounding and accreting into a black hole with a mass of about $10M_{\odot}$. The accretion disk is maintained by matter transferred from the binary companion of Cyg X-1, the blue supergiant HDE226868. The X-ray luminosity of Cyg X-1 is known to vary randomly on a wide range of time scales, from many days to a few milliseconds, with the shortest of these time scales corresponding to the dynamical time scale of the innermost stable orbit around the black hole^{1,2}. The optical luminosity of the Cyg X-1 system is also known to vary on time scales of days³, but only one case of extremely rapid optical variability has been reported^{4,5}. The properties of the rapid variations were most unusual. The variations were transient and were detected only four times, but they occurred sufficiently frequently that three of these detections occurred during the total of only 2 h of observations accumulated by Auriemma *et al.* in July 1975^{4,5}. The variations were nearly periodic, with periods of 83.53 ms, 83.71 ms, 83.59 ms, and with $|\Delta P/P| \sim 2 \times 10^{-4}$. The periodicities were long-lived, lasting at least 6 min and probably over 10 min, so that the pulse trains consisted of over 4,000 pulses. The amplitudes of the pulses were large, up to 0.042 mag. No comparable behaviour has been detected in the X-ray light curve of Cyg X-1, and it is far from clear that this behaviour is consistent with our present conceptions of accretion disks. Thus, the detections of these pulses, if confirmed, would be of some importance since they would require a revision of the models for Cyg X-1. Therefore, we have taken an extensive set of observations of Cyg X-1 with the purpose of detecting additional examples of the rapid periodicities.

The data were acquired using one or other of the high-speed photometers mounted on the 0.76 m, 0.92 m, or 2.08 m telescopes at McDonald Observatory. Our observational technique consisted of acquiring individual 10-min long light curves of HDE-226868. The light curves were taken through a wide variety of filters, most notably the Johnson U, B, and V filters, the Strömgen v filter, and a 30 Å filter centered on the $\lambda 4,686$ Å line of He II. Each of the light curves was tested for the presence of periodicities at the time it was observed. Our technique for testing for periodicities has already been described in detail⁶. Briefly, for a sinusoidal signal, the test is equivalent to calculating the power spectrum of the light curve, but because of limitations on computer memory and speed, the spectrum is calculated only for a restricted range of frequencies. For Cyg X-1, we chose a frequency range of 11.718 to 12.216 Hz. This frequency range is centred on, and is a factor of nearly 20 greater than the range of the frequencies detected by Auriemma *et al.* We accumulated over 90 h of light curves spaced over 65 nights in September to November 1976 and in May to October 1977. With the exception of a few observations heavily contaminated by clouds, the maximum amplitude of any possible periodic signal during this

time was 0.0029 mag, which is more than a factor of 10 less than the amplitude of the variations detected by Auriemma *et al.* With no exceptions, the power at all frequencies in the tested range was always consistent with white noise.

There are several ways to account for the difference between our results and those of Auriemma *et al.* Cyg X-1 could have changed its properties between 1975 and 1976 so that the periodicities are no longer present or occur far less often. The variations could have been present during our observations, but with periods outside the range of periods we tested. It is also possible that the periodicities detected by Auriemma *et al.* were spurious and of instrumental origin.

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Quasi-periodic fluctuations in electron content during a partial solar eclipse

CHIMONAS and Hines^{1,2} have suggested that a solar eclipse might generate gravity waves in the atmosphere. During solar eclipse the localised cooling of the atmosphere in the lunar shadow causes an energy imbalance and the shadow moving at supersonic speed across the Earth's surface could be a continuous source of gravity waves propagating to great distances in the atmosphere. These quasi-periodic wave perturbations in the ionospheric electron density, caused by the coupling between the ionised and the neutral particles, have been detected by various investigators³⁻⁵ at middle and high latitude stations away from the path of the eclipse shadow. Hajkowicz⁶ has reported the observation of perturbations of quasi-periods of less than 2 min, after the October 1976 eclipse. We report here the observation of quasi-periodic fluctuations in Faraday rotation angle Ω and the 1 MHz modulation phase delay ϕ of 40 MHz transmissions from ATS-6 geosynchronous satellite recorded at Trivandrum (dip $0^{\circ}57'S$, geographical longitude $76^{\circ}57'E$) during the partial solar eclipse on 29 April 1976. ϕ directly gives columnar electron density integrated along the radio ray path from the satellite to the receiver; whereas Ω gives columnar integrated electron density only up to an altitude of 2,000 km because of the weightage by the component of the geomagnetic field along the ray path. For ATS-6 to Trivandrum ray path geometry, a change of 10° in Ω will be produced by a change of 0.47×10^{18} el. m^{-2} of electron (el.) content. In contrast to this, 10° change in ϕ will be produced by a change of 0.34×10^{18} el. m^{-2} . The accuracy of measurement of both Ω and ϕ is better than 1° .

On 29 April 1976 the solar eclipse, as seen from the 300 km altitude point on the ATS-6 to Trivandrum ray path, began at 1718h (all times are IST); maximum obscuration of about 20% of the solar disk was at 1802 h; the end of the eclipse was at 1843 h and the sunset time was 2003 h. The values of Ω and ϕ recorded on 29 April 1976 between 1420 h and 1830 h were scaled at intervals of 2 min. The differences between the data and the 10-min moving averages which contain all fluctuations with quasi-periods less than 50 min are plotted against time in Fig. 1; separately for

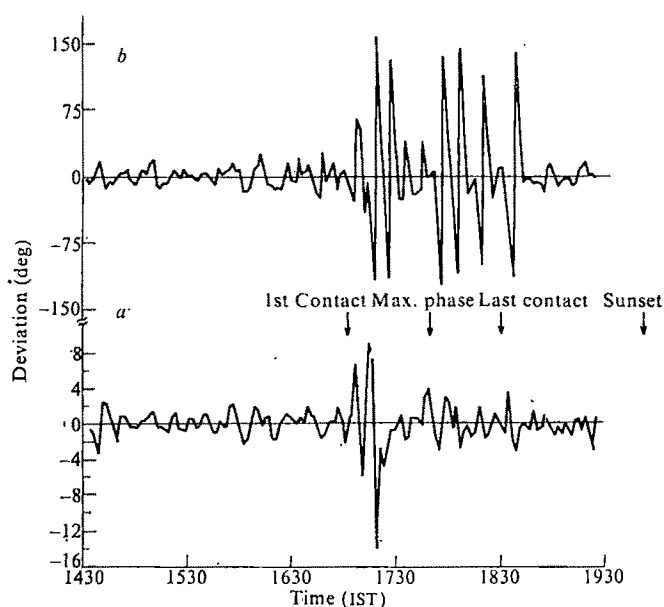


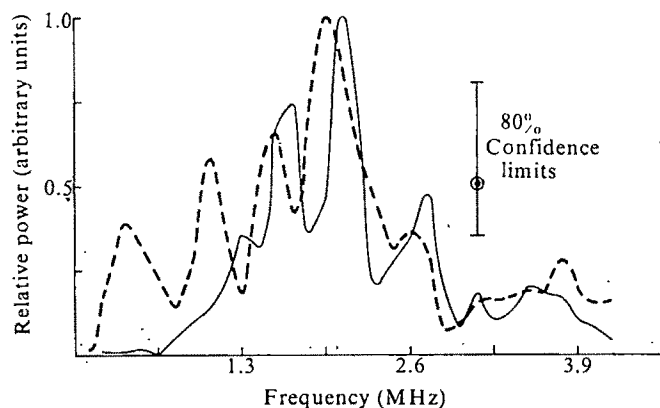
Fig. 1 Fluctuations of Ω (a) and ϕ (b) on 29 April 1976.

Ω (Fig. 1a) and ϕ (Fig. 1b). Figure 1 clearly shows fluctuations in both ϕ and Ω during the solar eclipse with quasi-periods around 11 min. A striking feature is the relatively quiet period of no fluctuations before and after the eclipse. The maximum amplitude of the total electron content fluctuations is estimated to be about 0.5×10^{16} el. m $^{-2}$ and this is about 1.0% of the total electron content. Similar analysis of the data between 1430 h and 2000 h on 28 and 30 April 1976 did not show any conspicuous quasi-periodic fluctuations. This strongly supports the view that the fluctuations shown in Fig. 1 during the solar eclipse period are in fact due to the solar eclipse.

From Fig. 1, it can be seen that there is not an exact one to one correspondence between the fluctuations of Ω and ϕ although, the ray paths along which they give the integrated electron content are the same. Also ϕ shows relatively clearer periodic fluctuations than Ω . This may be because Ω is weighted by the geomagnetic field.

To study the spectral components, the fluctuations of Ω and ϕ in Fig. 1, during the 128-min interval between 1652 h and 1900 h were subjected to power spectrum analysis with Hamming filter⁷. The power spectrum obtained has a frequency resolution of 0.13 mHz, the Nyquist frequency being 4.166 mHz. In Fig. 2 the continuous and the dashed curves are the power spectra of ϕ and Ω

Fig. 2 Power spectra of the fluctuations in Ω (dashed line) and ϕ (solid line) during the eclipse on 29 April 1976.



respectively. To test the stability of the spectral power estimates, the equivalent number of degrees of freedom of a χ^2 distribution⁷ have been computed and was found to be close to 22 for both the spectra. The 80% confidence limits of the relative spectral power estimate of 0.5 is shown in Fig. 2. The periods (in minutes) corresponding to the prominent spectral peaks in Fig. 2 are:

ϕ	—	12.8	9.8	8.0	6.1
Ω	42.7	16.0	10.7	8.5	6.5

It can be seen from atmospheric gravity wave theory⁸ that the above periods agree with the periods of gravity wave propagating modes at F-region levels. Considering the corresponding peaks in Ω and ϕ shown above it is immediately obvious that the periods are larger in Ω than in ϕ . This difference may be due to the influence of geomagnetic field on Ω .

The effect of the weightage of the geomagnetic field on Ω depends on the period and the vertical wavelength of the propagating gravity wave. For example, the electron density fluctuations at 350 km altitude produce about 50% smaller fluctuations amplitudes in Ω compared with the same electron density fluctuations at 200 km altitude. In Fig. 1, the amplitude of the fluctuations in ϕ is almost the same during the eclipse, whereas in Ω the fluctuations decreased to about 50% during the later part of the eclipse. This indicates that the fluctuations in electron density during the eclipse are not at the same height and that the fluctuations during the later part of the eclipse are at a higher altitude than soon after the eclipse beginning. A steady increase in the effective height of the electron density fluctuations in conjunction with the steady decrease in the geomagnetic field with height, leads to a larger period of the wave as seen on Ω than on ϕ . This upward energy propagation is in fact an important characteristic of the gravity waves.

In Fig. 2, the relatively larger spectral power at larger periods in Ω than in ϕ indicates that the larger period waves are propagating at lower altitudes, where for a given density change the change in Ω is larger because of the large value of the weighting geomagnetic field. This is also borne out by gravity wave theory⁸ that large period waves propagate at low elevation angles.

It can be seen that electron content fluctuations are shown better in ϕ than in Ω . The non-observation of clear fluctuations in electron content during earlier solar eclipses³⁻⁵ may be because all these measurements were of Ω fluctuations.

It is difficult to calculate the amplitude of the gravity wave produced by solar eclipse in the neutral particles and the consequent fluctuations in electron density by collisional coupling between the neutral and ionised particles. It is known⁸, however, that the gravity wave amplitude increases exponentially with height whereas the ion neutral collisional frequency decreases exponentially in the same way. This may perhaps lead to the constant amplitude of the fluctuation of ϕ in Fig. 1.

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Received 6 September; accepted 3 November 1977.

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A simple calculation of ozone depletion by chlorofluoromethanes using a two-dimensional model

THE possible perturbation of the total amount of ozone in the atmosphere due to man's activities has been the subject of much recent research. The vast majority of perturbation calculations have been performed using one-dimensional models in which variations in the vertical alone are considered. I describe here some simple calculations of the effects of chlorofluoromethanes on the ozone layer using a two-dimensional (latitude and height), time-dependent numerical model in which the mean circulation is computed at each time step. In our two-dimensional treatment the eddy transports of heat and matter are calculated using two-dimensional eddy diffusion coefficients, based on atmospheric statistics, and the eddy momentum fluxes are average monthly values for 1973 deduced from the Nimbus V Selective Chopper Radiometer measurements. The approach has proved successful in modelling many features of the unperturbed atmosphere and, in particular, of the behaviour of ozone in the stratosphere¹.

In the model the photochemical source of ozone is linearised such that

$$\frac{\partial [O_3]}{\partial t} = \frac{[O_3]_e - [O_3]}{\tau}$$

where $[]$ represents a mass mixing ratio, $[O_3]_e$ is the ozone equilibrium mixing ratio and τ the time constant. $[O_3]_e$ and τ are specified as functions of latitude, height and season, the values being appropriate to a photochemical scheme including the oxides of nitrogen and hydrogen. The solar heating is dependent on the modelled ozone concentrations, thus coupling the chemistry, radiation and dynamics. Further details of the model are given in refs 1 and 2.

A complete calculation of the effect of chlorofluorocarbons on ozone would require simulation over many years. The purpose of this letter is not to predict the magnitude of these effects, however,

Fig. 1. The latitude-time section of the percentage change in total ozone between experiments E1 and E2 for one model year. Model days 730–1095.

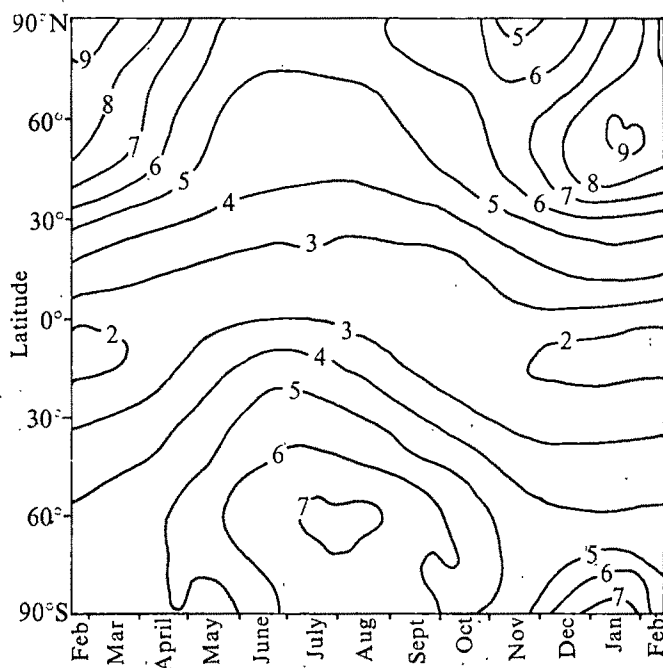
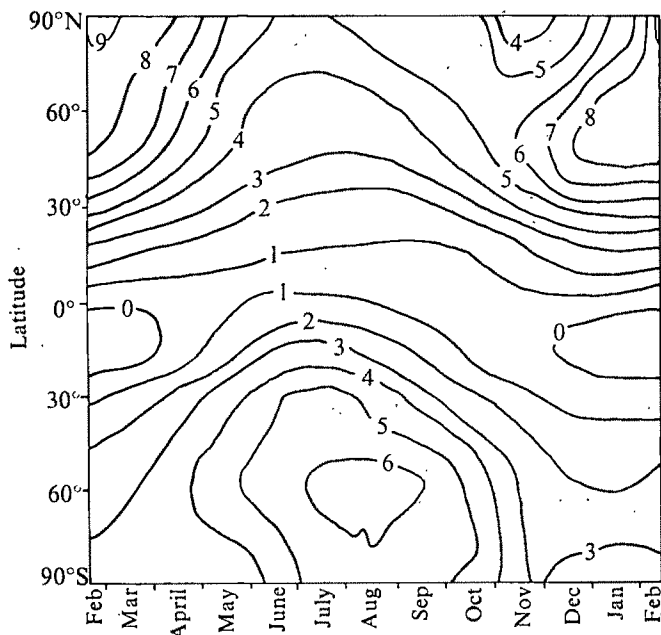


Fig. 2. The latitude-time section of the percentage change in total ozone between experiments E1 and E3 for one model year. Model days 730–1095.

but rather to indicate that there are important latitudinal and seasonal variations which need to be considered. For this reason it seems justifiable to make some rather gross approximations. The starting point for the study is the work of Crutzen³. Using a one-dimensional, steady-state model, he calculated the reduction in ozone due to continued release of chlorofluoromethanes at a rate appropriate to the mid-1970s. Large changes are predicted for the upper stratosphere where ozone is expected to be close to photochemical equilibrium.

The results of three different calculations will be described here: E1, an unperturbed run very similar to that described in Harwood and Pyle¹; E2, a perturbed run in which the equilibrium values are modified, in a manner based on Crutzen's work as described below, and which uses exactly the same temperature and wind fields as in E1; E3, an experiment in which the equilibrium values are again modified but in which the perturbed ozone concentrations are used in the calculation of solar heating. Thus, the feedback between the ozone amount, temperature and the mean circulation is included in E3.

In all three runs the ozone equilibrium concentrations are taken to be temperature dependent according to the expression $[O_3]_e(T) = A \exp(1,000/T)$ (see Barnett *et al.*⁴). The results of E1, including this temperature dependence, are similar to those described by Harwood and Pyle¹.

In the perturbed runs the equilibrium values were modified in the following manner. The stratospheric values of $[O_3]_e$ were modified by the percentage calculated by Crutzen³ (table 1 ref. 3). τ , too, depends on $[O_3]_e$ as well as on the concentration of chlorine compounds and the dissociation rate of ozone. Allowance was made for all these factors in E2 and E3.

Figure 1 shows the latitude-time section of the percentage difference in total ozone between E1 and E2. The magnitudes clearly depend on the initial assumptions and should not be emphasised. What is of interest is the large variation with latitude and time. The largest reductions occur in high latitudes where the total ozone itself is largest and at these latitudes the reductions vary by a factor of two with the time of year. In equatorial regions, where total ozone is lowest, there is even an increase in the total ozone throughout part of the year. This is due to the 'self healing' effect whereby increased penetration of solar radiation in the lower stratosphere increases ozone there. Ozone is produced in the equatorial lower stratosphere and transported in the upward branch of the Hadley cell to regions where it has been depleted.

The increased upward transport in E2 can, at certain times of the year, cancel the reductions at higher levels.

The above result is due to a combination of dynamical and photochemical processes. If a climatological ozone distribution were reduced by the same factors as $[O_3]_e$, the largest percentage reduction in total ozone would be found in equatorial latitudes. On the other hand, the percentage reduction of the equilibrium values between E1 and E2 does have largest depletion in high latitudes but in this case the magnitudes differ considerably from those in Fig. 1.

In E3 the reduced ozone concentrations are used in the solar heating calculations in the model. One might expect that reduced ozone in the upper stratosphere would lead to reduced solar heating and lower temperatures there. The ozone, though, is temperature dependent; lower temperatures cause the ozone amounts to increase. Hence, the mean circulation, driven in part by the solar heating, differs from that in E1 (and E2, where this feedback was deliberately suppressed). Figure 2 presents the latitude-time section of the percentage difference in total ozone between E1 and E3 and has many similarities with Fig. 1. Surprisingly, the reduction of ozone in E3 is larger than in E2. The stratospheric temperatures in E3 are reduced by a few per cent and the ozone reduction at this level is correspondingly less than in E2. In the mid and lower stratosphere in E3, however, there is an increase in temperature and a decrease in ozone when compared with E2 and this more than cancels the upper stratospheric increase. Thus, for instance, in E3 there is no longer a small increase in total ozone at the equator. That the temperatures in the lower stratosphere are higher in E3 is probably due not only to increased solar heating but also to transport processes. It is evident from E3 that the calculation of ozone reduction is made a more complicated exercise by these important feedbacks between photochemistry, radiation and dynamics.

The results presented here demonstrate the importance of multidimensional models in studies of projected perturbations in atmospheric ozone. An extremely simple photochemical scheme has been used here and there are strong reservations about the absolute magnitude and phase of the calculated reductions in ozone. But, both E2 and E3 demonstrate, unequivocally, important variations in ozone depletion with both latitude and time (and this is confirmed by preliminary results with a model including a much more complete photochemical scheme, data not shown). Furthermore, E3 indicates the importance of including all the important feedback mechanisms.

This work was supported by the Department of the Environment.

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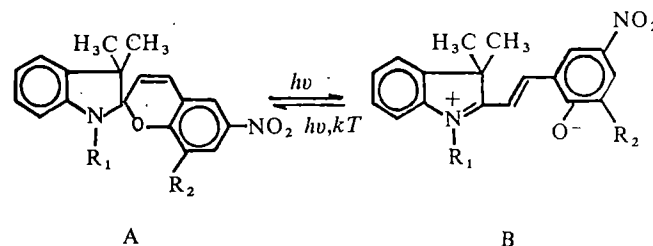
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Quasi-crystals from irradiated photochromic dyes in an applied electric field

In many photochromic systems, and certainly in the spiropyran, the colourless (A) form is much less polar than the photocoloured (B) form¹. Our previous experiments^{2,3} revealed unusual properties of spiropyran in non-polar solvents at low temperatures (173–240 K). On irradiation of these solutions with suitable light there were formed dimers (AB) and charge transfer complexes (CTC $\equiv A_n^+B^-$ with $n \approx 2-3$), and these in turn formed aggregates with degrees of association $>10^8$. The CTC exhibit spectral red shift of about 100 nm as compared to the dimers. The



relative populations of dimers and charge transfer complexes in the aggregates depended both on light intensity and temperature, because CTC formation involves an activation energy of about 5 Kcal mol⁻¹ associated with the interaction of dimers with further A molecules. At temperatures sufficiently low or at light intensities sufficiently high it was possible to obtain aggregates composed of dimers only. When the constant field was applied during irradiation, threads were formed which extended from one electrode to the other (along the electric lines of force). These threads are composed of dimers and CTC, and their absorption spectrum is identical to that of aggregates in solution. Linear dichroism measurements showed that the CTC are oriented along the thread axis, but the dimers are unoriented. These threads were formed only in the

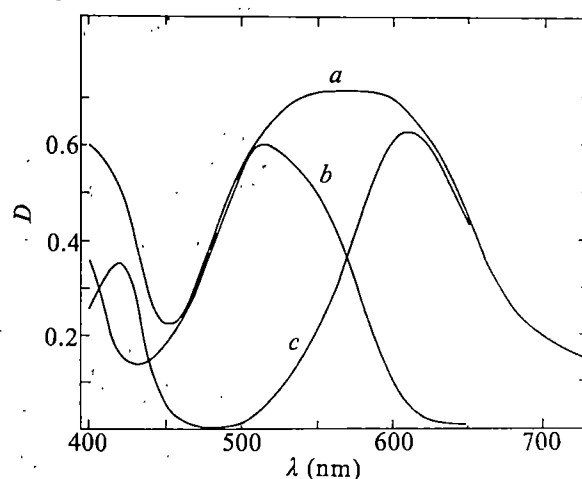


Fig. 1 The absorption spectra of 6-nitro BIPS (concentration in methylcyclohexane solution 5.10^{-4} M), irradiated by ultraviolet light with intensity of 2.10^{-8} einstein cm^{-2} s. *a*, Quasi-crystals, room temperatures, $F = 15 \text{ kv cm}^{-1}$; *b*, aggregates -120°C . *c*, Difference spectrum (absorption of the CTC).

temperature range in which CTC arise in solution. Here we describe investigations of the structure of such threads, which led us to conclude that the threads consist of highly dipolar crystallites composed of the charge transfer complexes and coated by an amorphous phase composed of dimers. These crystallites in the amorphous envelope are oriented along the electric field and joined together to give the quasi-crystalline threads.

Solutions of 6-nitro-1',3',3'-trimethylspiro[2H-1-benzopyran-2,2'-indoline, '6-nitro BIPS' ($R_1 = \text{CH}_3$; $R_2 = \text{H}$) (ref. 4), in methylcyclohexane were studied. An Osram HBO 200 W high pressure mercury lamp in a thin housing was used for irradiation, with Corning glass filter No. 5840 which transmits essentially from 300 to 400 nm. The absorption spectra were measured using a Cary 14 spectrophotometer, and the linear polarised spectra using the same instrument with the attachment described by Jaffe *et al.*⁵. This attachment permits simultaneous measurement of absorptions in two mutually perpendicular directions. This proved useful in the present experiments because the

Table 1 Degree of linear dichroism of the threads from 6-nitro BIPS in methylcyclohexane

Concentration of solution M $\times 10^3$	Applied field kV cm ⁻¹	P_{310} (± 0.03)	P_{010} (± 0.02)
3.0	5.0	0.03	0.11
3.0	7.5	0.02	0.09
1.5	25	0.00	0.1
0.8	25	-0.03	0.11
0.5	25	-0.03	0.12
0.4	25	-0.01	0.12

$P_\lambda = (D_{||} - D_\perp)/(D_{||} + D_\perp)$; $D_{||}$ and D_\perp are the optical densities of the threads when the monitoring light is polarised, respectively parallel and perpendicular to the axes of the threads.

threads are rather unstable. But the method limited us to measurements of absorption in specific directions only, that is parallel and perpendicular to the threads axis.

We used spiropyran solutions of higher concentrations ($>10^{-4}$ M) and higher light intensities ($>10^{-8}$ einstein cm⁻² s⁻¹) than in the earlier studies^{2,3}. This permits the production of much higher stationary concentrations of form B and the growth of the quasi-crystals even at room temperature.

Figure 1 shows the spectrum of quasi-crystals obtained from 6-nitro-BIPS at room temperature in an electric field. This spectrum is identical to that of aggregates obtained under the same conditions in the absence of a field. Also the spectrum of aggregates obtained at 153 K in solution is shown, which consist of dimers only without CTC. The difference spectrum, constructed as described previously³, is the absorption due to CTC; this is shown in Fig. 1c. This absorption is red-shifted by about 100 nm with respect to the absorption of the dimers.

The linear dichroism, P_λ (see Table 1) was measured for various initial concentrations of the solution and for various strengths of the applied field. The results show that P_{310} , determined near the maximum dimer absorption, is close to zero; on the other hand, P_{010} , determined near the maximum CTC absorption, is equal to about 0.1, and does not vary with the concentration of the photochrome or the field strength (5–25 kV cm⁻¹).

We shall show that the threads consist of small crystallites of CTC embedded in dimers. The dichroism results then show that there is a random arrangement of the dimers in the threads; on the other hand, the microcrystals in the thread are all aligned, and in such a way that the absorption by the CTC of the light polarised parallel to the thread axis exceeds, although slightly, the absorption of the light polarised perpendicular to this axis. This orientation of the microcrystals must be due to the interaction $F\mu$ between the field of strength F and some permanent dipole moment μ , with $F\mu > kT$. This inequality is satisfied at 293 K and for $F = 5$ kV cm⁻¹ when $\mu > 2.5 \times 10^{-30}$ D. This corresponds to a dipole length $>10^{-5}$ cm which considerably exceeds the size of the complexes $A_n^+B^-$ ($n = 2-3$). The conclusion is that it is associates or microcrystals of CTC rather than the individual CTC which are oriented by the applied field.

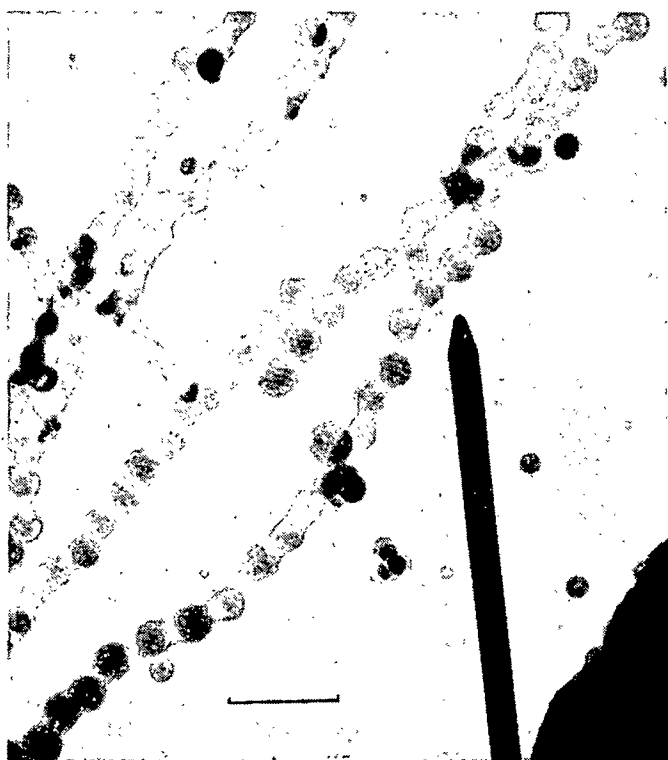
We recall that the photocoloured B form of the spiropyrans are merocyanines which readily aggregate in solution to give stacks with a tilted 'deck-of-cards' structure⁶. The absorption of such aggregates are red-shifted by about 100 nm as compared to the absorptions of the corresponding unaggregated molecules. In our case, the stacks as well as the crystals which are formed from the highly dipolar CTC may or may not be dipolar depending on their structures. Our evidence points to the crystals being oriented by the field. Thus we conclude that the crystals are dipolar. Since our measurements of polarised absorption were limited to directions parallel and perpendicular to the thread axis, we

cannot tell whether the observed value of P_{010} is its maximum value, and cannot ascertain the relationship between the orientations of the CTC chromophore and the crystal dipole moment.

Additional structural information has been obtained by X-ray and electron diffraction studies. The X-ray powder patterns of precipitates formed at room temperature in the absence of an applied field and of the quasi-crystalline threads are practically identical; they consist of sharp reflections, the most intense ones corresponding to interplanar spacings of 3.4, 5.0, and 7.4 Å. These sharp reflections are superimposed on a diffuse pattern, the intensity of which rises with increasing dimer content. Thus precipitates formed at low temperature (150 K), which are composed of dimers only, with no CTC, give only the diffuse pattern. Therefore both the precipitates and the quasi-crystals are composed of various proportions of dimers, in a more-or-less amorphous state, and of crystalline CTC.

Electron microscope studies of precipitates formed in the absence of a field show them to consist of globules, $\sim 0.2-0.4$ μ m in thickness. For the electron microscope studies of the threads the latter were produced directly on a parlodion film, carefully dried, and transferred to grids. In the micrograph (Fig. 2) one can see similar globules of those of the precipitates, joined together by straight, narrow portions of the threads, which also prove to be amorphous. Because of the thickness of the globules it is not possible to get distinct electron diffraction patterns from their central parts, but such patterns can be obtained sometimes by focussing on the edges. The patterns thus obtained consist of the reflections from one or more single crystals. The diffraction from the crystals can be readily detected in those cases in which the amorphous envelopes of the globules have been (accidentally) removed. We conclude that crystals are contained in the globules, and that their dimensions are of the same order of magnitude as estimated from spectroscopic data. Further, since limiting dichroism is achieved even at the low field-strength of 5 kV cm⁻¹, the microcrystals seem to be aligned parallel in the threads.

During irradiation of spiropyrans, in non-polar solvents

Fig. 2 Electron micrograph of the quasi-crystals. Scalebar, 1 μ m.

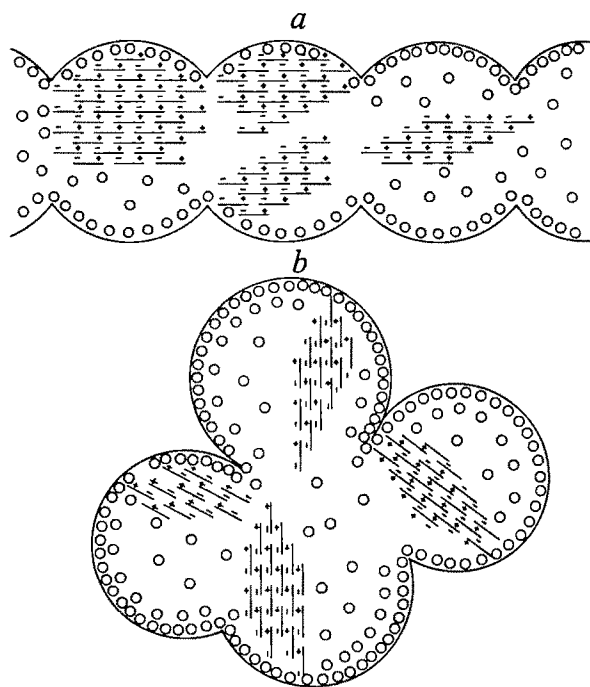


Fig. 3 Scheme of structures of quasi-crystals (a) and aggregates (b).

dimer and charge-transfer complexes are formed. These give solids consisting of amorphous zones of dimer and dipolar crystals of the complexes. In an electric field the crystals coated with dimer are joined together to give quasi-crystalline threads in which the crystals are oriented with their dipolar axes along the lines of force and thread axes. These threads thus form a new, linearly organised form of organic material (Fig. 3).

We thank Professors M. D. Cohen and E. Fischer, and Dr E. Wachtel, for valuable discussions, and Mr M. Kaganowich for the spiropyran.

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Received 23 August; accepted 3 November 1977.

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Rare gas isotopic compositions in diamonds

RARE gas isotopic compositions such as $^{40}\text{Ar}/^{36}\text{Ar}$, $^3\text{He}/^4\text{He}$ and $^{129}\text{Xe}/^{132}\text{Xe}$ in the Earth have provided a powerful tool for understanding the origin and evolution of the terrestrial atmosphere¹⁻⁴. The isotopic information may be obtained from rare gases trapped in some mantle-derived materials such as volcanic rocks, volcanic xenoliths or volcanic gases. Among these mantle-derived materials, diamond seems to be unique due to its almost complete inertness to any known chemical and to its enormous stability against high temperature. Although the presence of O_2 , H_2 , CH_4 , H_2O , CO , N_2 , Ar and CO_2 in diamonds has been

reported^{5,6}. No previous measurement has been made either on elemental compositions or on isotopic ratios of rare gases in diamonds. Here we report on rare gas elemental composition and isotopic ratios in diamond. We found that $^3\text{He}/^4\text{He}$ ratio is more than an order of magnitude larger than the atmospheric value and also $^{40}\text{Ar}/^{36}\text{Ar}$ ratio is significantly higher.

Industrial diamonds we studied are believed to have come from Kimberley Mines, South Africa. The size of the diamonds ranges from about 1 mm to about 5 mm. Some contain black inclusions, some of which are ferromagnetic and appears to be pyrrhotite under a microscope. Existence of pyrrhotite inclusions in diamond is not unusual⁷ and the black inclusions seem to be syngenetic with diamonds. Diamonds are crushed to a few meshes in a stainless steel mortar. Step-heating was made on two batches of samples at 800 °C, 2,000 °C and 2,100 °C in a high vacuum tantalum furnace⁸. Only visible difference between the two batches is that batch 1 is richer in black inclusions than batch 2.

Rare gas elemental abundance and their isotopic ratios are shown in Table 1. Batch 1 gave almost three times more rare gases as batch 2. Also isotopic ratios, $^{40}\text{Ar}/^{36}\text{Ar}$ and $^3\text{He}/^4\text{He}$, of the two batches are quite different. We believe that this is due mainly to difference in the amount of the black inclusions, that is, rare gases in batch 1 may come mostly from the black inclusions, while those in batch 2 are more representative of diamonds. In both cases major degassing is observed at 2,000 °C. A third batch of sample which were heated at 1,500 °C gave off $^{40}\text{Ar} \sim 1 \times 10^{-8} \text{ cm}^3 \text{ g}^{-1}$ at STP which is less than 10% of the rare gases evolved from batch 1 and 2 at 2,000 °C. X-ray analyses on heated samples showed that heating at 1,500 °C for 1 h (batch 3) did not result in graphitisation, but heating at 2,000 °C for 1 h (batch 1 and batch 2) completely converted diamonds to graphite. We conclude, therefore, that graphitisation which accompanies any density change of more than 30% must be responsible for the major degassing of diamonds.

All other rare gases except He, show a similar thermal release pattern. In the case of He, more than 99% was degassed at 2,000 °C suggesting that most of He resided at low temperature sites had been degassed before the experiment, perhaps under high temperature mantle condition. From the similar thermal release pattern of rare gases between batch 1 and batch 2, we conclude that rare gases in the black inclusions occupy similar sites as those in batch 2, the latter being more likely to represent diamond rare gases. This suggests a syngenetic origin of the black inclusions with diamonds.

The K content of evaporates which were deposited on the inner wall of a high vacuum extraction furnace during the graphitisation were measured. The deposits were washed by dilute HNO_3 and the K content in the washed solution was measured with an isotope dilution method. The amount of potassium was 8.22 p.p.m. and 1.97 p.p.m. for batch 1 and batch 2 respectively. Radiogenic ^{40}Ar , which would have been produced in batch 1 diamonds by this amount of K, will exceed the total amount of ^{40}Ar if the age of the diamonds is older than 2,800 Myr. Hence, the diamonds can not be older than 2,800 Myr. If this maximum age is assumed, this will give minimum $^{40}\text{Ar}/^{36}\text{Ar}$ ratio of 670 for the trapped Ar in the batch 2 diamonds. The observed $^3\text{He}/^4\text{He}$ ratio is also minimum, as the diamonds must contain some U as well as K. Therefore, the $^3\text{He}/^4\text{He}$ and $^{40}\text{Ar}/^{36}\text{Ar}$ in the diamonds must be larger than 1.95×10^{-5} and 670. The $^3\text{He}/^4\text{He}$ ratio is significantly higher than those observed in ridge submarine glasses⁹ which are generally regarded to be derived from oceanic upper mantle. The high $^3\text{He}/^4\text{He}$ ratio suggests that the diamonds may be derived from a region different from source regions for oceanic ridge basalts, most likely from deeper region in the mantle. In this connection it is interesting that similar high $^3\text{He}/^4\text{He}$ (2.09×10^{-5}) are observed in Hawaiian volcanic gases⁹, which may be derived from deeper mantle through a hot spot.

Although the origin of diamonds is not fully understood, it is generally assumed that they crystallise from CO_2 saturated fluid phase in the mantle, probably deeper than 100 km or even more. The syngenetic black inclusions occluded in diamonds seem to have been derived from mantle region in which diamonds crystallised. Our results lead us to an interesting speculation that

Table 1 Elemental and isotopic compositions of rare gases in diamond

Sample	Batch 1			Blank 1		Batch 2			Blank 2
Weight (g)		1.98				2.61			
Temperature (°C)	800	2,000	2,100	2,000	800	2,000	2,100	2,000	
Time of heating (min)	30	60	5	60	30	60	60	60	
³ He × 10 ⁻¹³ cm ³ g ⁻¹ (STP)	<3	286	<10	<20	<10	179	<10	<20	
⁴ He × 10 ⁻⁹	1.36	3480	0.09	1.1	1.57	918	0.37	2	
²⁰ Ne × 10 ⁻¹²	6.3	20.7	< 0.1	20	2.1	10.2	< 0.1	20	
³⁶ Ar × 10 ⁻¹¹	3.35	36.7	10	2.4	1.54	9.37	1.90	2	
⁸⁴ Kr × 10 ⁻¹²	1.8	3.6	< 0.02	0.6	1.0	2.9	0.26	0.8	
¹³² Xe × 10 ⁻¹³	3.5	9.1	< 2	2	4.4	7.3	1.0	2	
³ He/ ⁴ He	<2 × 10 ⁻⁴	(8.23 ± 0.35) × 10 ⁻⁶				(1.95 ± 0.07) × 10 ⁻⁵			
⁴⁰ Ar/ ³⁶ Ar	359 ± 2	436 ± 2			574 ± 14	1,121 ± 8			
¹²⁸ Xe/ ¹³² Xe		0.0754 ± 0.0011				0.0718 ± 0.0037			
¹²⁹ Xe/ ¹³² Xe		0.996 ± 0.019				0.978 ± 0.004			
¹³⁰ Xe/ ¹³² Xe		0.161 ± 0.007				0.149 ± 0.006			
¹³¹ Xe/ ¹³² Xe		0.786 ± 0.023				0.779 ± 0.014			
¹³⁴ Xe/ ¹³² Xe		0.391 ± 0.012				0.386 ± 0.009			
¹³⁶ Xe/ ¹³² Xe		0.328 ± 0.012				0.318 ± 0.018			

Other isotopic ratios are indistinguishable from atmospheric values

rare gases observed in batch 2 may be representative of those carried by CO₂ phase, whereas rare gases in batch 1, or more specifically rare gases in the black inclusions, is rather indicative of a region where the diamonds occluded the former.

Ar isotopic ratios observed in diamonds do not seem to support the speculation¹⁰ that diamonds were formed from materials in subducting ocean sediments, since even very small amount of seawater contamination (say less than 0.1%) which would necessarily accompany ocean sediments would reduce ⁴⁰Ar/³⁶Ar isotopic ratio to almost atmospheric value (295.5) because of relatively very high Ar content (about 0.03%) in seawater. The observed ⁴⁰Ar/³⁶Ar is much higher than the atmospheric ratio, ruling out any noticeable amount of seawater contamination.

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Received 2 August; accepted 4 November 1977.

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On melting icebergs

THE feasibility of towing icebergs to their coasts and melting them on arrival to provide a supply of fresh water is being studied in various parts of the world. Saudi Arabia, Australia and California¹ are amongst those considering such a project. One suggestion for melting the icebergs is to run each iceberg aground in water approximately 250 m deep, the mean depth of Antarctic icebergs. A relatively shallow pen would then be built around the iceberg and it is conjectured that the melt water will rise, without much mixing, into the pen, from where it will be siphoned off for subsequent use. Neshyba² claims, however, that melt water produced by icebergs may be responsible for a large amount of upwelling and mixing in the Antarctic's Weddell Sea. He asserts that as the melt water rises up the side of an iceberg, it entrains a sizeable quantity of warmer, saltier water from

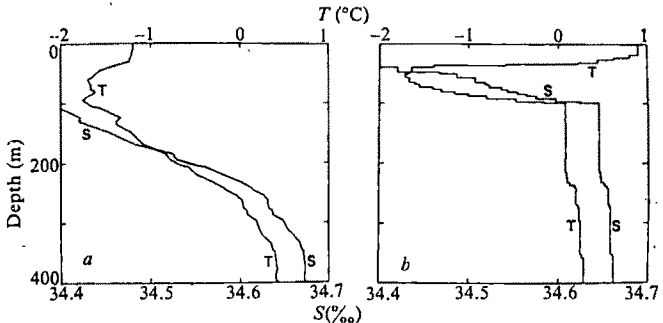


Fig. 1 Temperature (T) and salinity (S) profiles measured in the upper 400 m of the Weddell Sea (adapted from Fig. 3 of Foster and Carmack³). a, Near the Scotia Ridge at the northern edge of the Sea; b, near the turning point of the current gyre.

the environment. Neshyba calculates that an average-sized iceberg is thus responsible for a vertical volume transport into the upper layer of the Weddell Sea of 3.3×10^8 cm³ s⁻¹. This is a considerable transport, and the raising of water and nutrients from depth by this mechanism would be of importance in determining the physical and biological properties of the Weddell Sea. The first suggestion, that fresh melt water rises to the surface, and Neshyba's claims that the melt water will have mixed significantly with its salty environment before reaching the surface, cannot both be correct. We show here that both may be in error.

The vital fact neglected in both arguments is that the ocean environment is not only salty, but there is also a salinity gradient in the upper 250 m of most areas of the oceans. For example, data from the Weddell Sea shown in Fig. 1 (taken from Foster and Carmack³) indicate that the salinity increases with depth, as does the temperature in this region. The data indicate further that both the salinity and temperature distribution have a characteristic 'stepped' structure, with layers of well mixed temperature and salinity separated by sharp interfaces in which both properties vary more rapidly. Similarly, off the Californian coast the salinity again has a general increase with depth, while there is an overall decrease in temperature (see, for example, Gregg and Cox⁴, in particular their Fig. 1). We have carried out a series of experiments in which ice is inserted into a salinity gradient, and the preliminary results are reported here.

Iceblocks made from water which had been deaerated under vacuum measuring approximately 20 cm × 10 cm × 3 cm were inserted vertically (see Figs 2-3) into salt-stratified water at room temperature (~23 °C) with constant density gradients of between 1×10^{-3} and 6×10^{-3} g

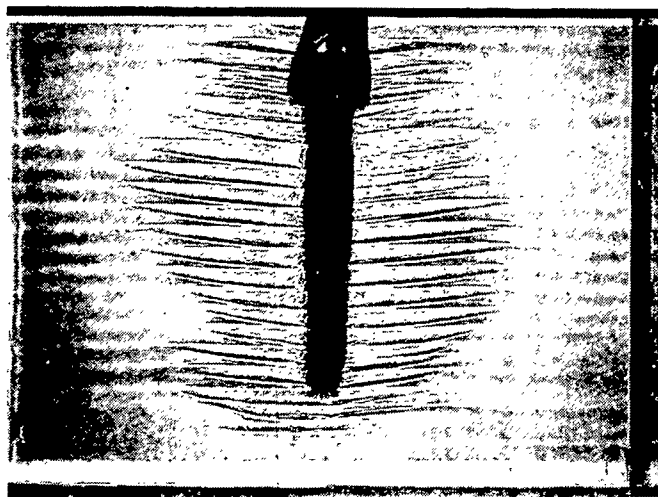


Fig. 2 Shadowgraph photograph showing the tilted layers and interfaces produced by inserting a block of ice into salt-stratified water at room temperature. (Specific gravity at top of tank 1.00, and at bottom 1.05; depth 26 cm.)

cm^{-3} per cm and surface densities between 1.00 and 1.10 g cm^{-3} .

Adjacent to the ice, melt water at 0°C rises in a thin, generally turbulent boundary layer until its density equals that of the surroundings at the same level. Above the level where these densities are equal the melt water in the boundary layer sinks. In our experiments this level occurs at a density of approximately 1.01 g cm^{-3} (if this exists), although in the ocean, with its typically lower temperature and higher salinity, such a level (where the density of the fresh melt water equals that of its surroundings) is unlikely to occur. The presence of a horizontal temperature gradient imposed upon the existing vertical salinity gradient alters the boundary layer a small distance away from the ice, producing a regular series of tilted convecting layers which grow out into the environment, as shown in Figs 2–4.

These layers exist because of double-diffusive convection, which readily occurs whenever two components of different molecular diffusivities contribute to the density. The general principles governing this form of convection and some applications are discussed by Turner³. Along the bottom of each layer, relatively fresh, cold water acquires heat and some

Fig. 3 An experiment carried out using the same conditions as shown in Fig. 2, but with fluorescein frozen into the ice block, and side illumination. The spread of the dye again shows the layers, but also indicates the distribution of the melt water.



salt across the 'diffusive' interface separating it from the environmental water at the top of the layer below. Thus the melt water becomes progressively lighter and runs uphill as indicated by the upward tilt of the layers seen in Figs 2–3. Along the top of the layers there is an inflow of environmental fluid, which is directly mixed with the melt water only near the ice. In this way a considerable portion of the melt water can be fed into the environment close to the level at which it is produced and very little, if any, rises to the surface.

The melting rate of the ice is controlled by the rate of heat transfer from the environment, and is thus greatly influenced by the circulation that occurs. On a large scale this means that increasing the salinity of the environmental fluid increases the relative buoyancy of the melt water.

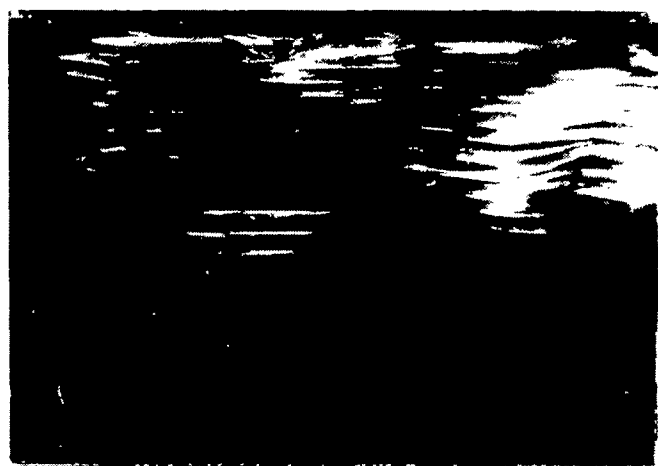


Fig. 4 The experimental tank at a later stage of the experiment of Fig. 3, soon after the iceblock had been carefully removed. The volume of dyed water is much greater than that of the ice block, indicating that the melt water mixes with a large volume of salty water and spreads out close to the level at which it is produced; little reaches the surface.

The velocity in the boundary layer thus increases, which results in increased melting. On a smaller scale, the layered motion produces a series of horizontal ridges (see Fig. 5) along the sides of the ice, with the deepest erosion corresponding to the centre of each of the layers, and the ridges to the interfaces between them. In each experiment, the layer size is reasonably uniform with depth and increases with either decreasing density gradient or increasing central salinity. In the laboratory this size is of the order of 1 cm. In the Antarctic, the weaker salinity gradients will tend to make the layers deeper, though this will be counteracted by the smaller horizontal density gradients. The quantitative scaling relations are to be discussed elsewhere.

Further suggestions follow immediately from the results of these experiments. First, the data displayed in Fig. 1b were obtained near the centre of the large gyre which characterises the circulation in the Weddell Sea. This structure differs markedly from that at the edges of the gyre (Fig. 1a) in that it has much stronger temperature and salinity gradients from 50–100m depth, weaker density gradients from 50–500 m and a more clearly defined step structure. We conjecture that this difference is due to the systematic injection of melt water at intermediate depths by icebergs which spend a longer time near the centre of the gyre. The spatial variability of the layering in this region³ is consistent with such a localised mechanism of formation, rather than a more extensive 'one-dimensional' convection process acting over the whole area.

Further measurements of temperature and salinity, made as close as possible to an iceberg in order to detect the layering, would be instructive. It would then be worthwhile



Fig. 5 An oblique view of a melting ice block, taken through the water surface. Note the ridges, caused by the uneven melting associated with the convection in layers.

exploring the structure of the layers as a function of distance from the ice. A direct search for the ridges associated with the variation in rate of melting produced by the convection might also be considered.

Finally, our results imply that those seeking to obtain fresh water from icebergs will have to consider alternative methods which isolate the melt water in some way from the surrounding seawater.

We thank R. Wylde-Browne for his able assistance with the experiments and particularly with the photography. This research was carried out while H.E.H. was a visiting fellow at the Australian National University and was in part supported by grants from the Royal Society and the British Admiralty.

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Received 21 September; accepted 3 November 1977.

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Iceberg sounding by impulse radar

KNOWLEDGE of an iceberg's draft is essential for assessing its risk to underwater installations, in predicting its drift, and for estimating its total bulk. Because of the highly irregular shape of icebergs, it is impossible to estimate an iceberg's draft directly from its above-water dimensions. Large tabular icebergs have been sounded using radio techniques^{1,2}. We report here that estimates of the draft of irregularly-shaped icebergs can also be obtained from the air quickly and accurately using short-pulse radar. A small iceberg in Twillingate Harbour, Newfoundland (49° 40'N, 54° 46'W) was sounded from a helicopter using impulse radar, on 11 June 1977. The result was verified by simultaneous measurement of the iceberg's draft using side-scan sonar.

The impulse radar equipment³, built by Geophysical Survey Systems consists of a control unit and an FM tape recorder, which were mounted in a helicopter, and a transmitter-receiver and antenna assembly, which was slung in a net about 6 m beneath the aircraft. The radiation pattern of the antenna is similar to that of a half-wave dipole. The transmitted signal is a broad-band pulse with a duration of about 20 ns and a centre frequency near 80 MHz. The receiver was set to accept echoes for up to 1.4 μ s, and by sequential sampling of the received signal, an audio-frequency replica trace was constructed and recorded on tape. After sampling, the effective repetition rate was 51.2 scans s⁻¹.

One of the impulse radar records is shown in Fig. 1, taken with the aircraft flying approximately 20 m above sea level. Strong echoes are represented by dark lines; signals received with long delay time are enhanced by a time-gain amplifier. Initial returns are due to reflections of the transmitted pulse between the antenna and the helicopter. The echo from the sea surface is very clear, but, as the iceberg is approached, this echo fades and a faint return from the sharply-peaked top of the iceberg is seen. Reflections from within the iceberg are very clear, although there is no unique bottom echo, since the radar signal reverberates within the iceberg. A set of multiple echoes is received with longer delay time.

To interpret the iceberg draft, the speed of the radar pulse in ice must be known. The speed of electromagnetic waves travelling in a dielectric medium is given by $c\epsilon_r^{-1/2}$, where c is the speed of light in free space, and ϵ_r is the relative dielectric constant of the medium. A dielectric constant of 3.2 was used, giving a velocity of 0.084 m ns⁻¹ of two-way travel time in ice. Ice samples collected from the iceberg surface were polycrystalline, and had a density of 0.938 ± 0.011 Mg m⁻³. These values for density and dielectric constant are consistent with previous work³.

The travel time used to interpret the draft was that from the first subsurface echo that was received directly beneath the centre of the iceberg. This return represents the most direct route through the ice, and it had a consistent multiple. Of course, there is no guarantee that this return came from the deepest subsurface point of the iceberg. The average draft of the iceberg was estimated to be 18.0 ± 0.9 m, with a standard deviation of 0.7 m over 16 passes. The height of the iceberg was also estimated, giving a draft: height ratio of about 4.3:1.

The hyperbolic patterns seen on either side of the iceberg are interpreted as reflections from pulses which entered the upper side of the iceberg, and traversed the iceberg before returning. Their path length in the ice was greater than the direct top-to-bottom path length, and the hyperbolic pattern is caused by the increased path length in the air as the horizontal helicopter-iceberg separation increased. These hyperbolic returns are often stronger than the direct bottom return as a larger proportion of the signal incident on the

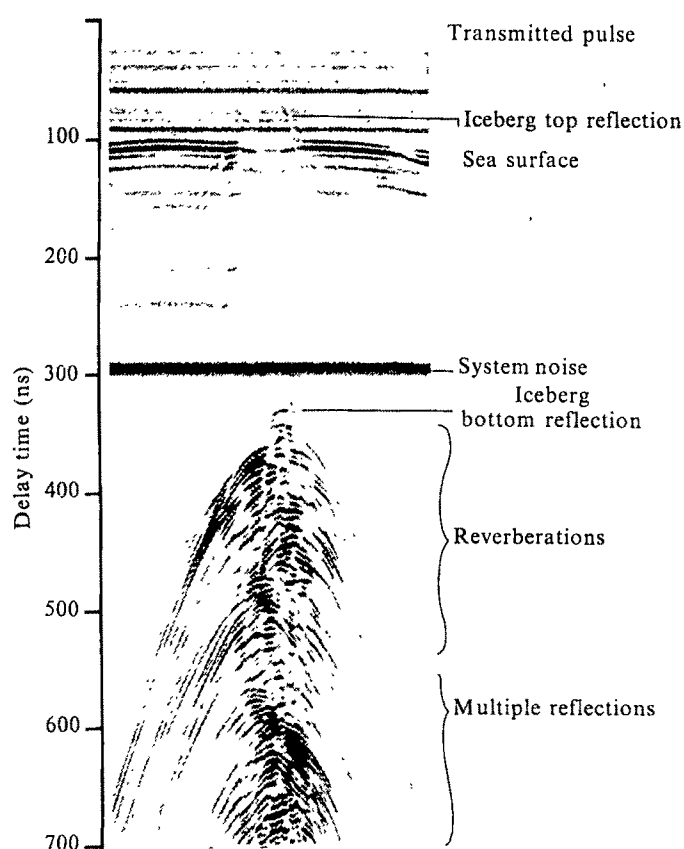
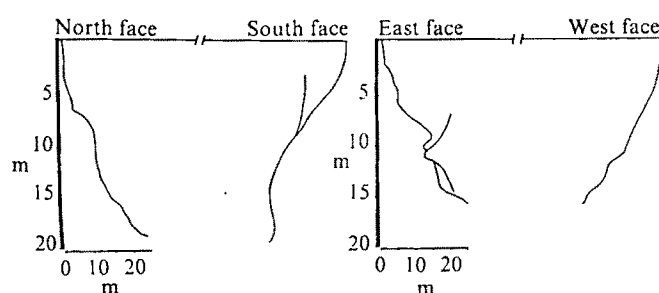


Fig. 1 Impulse radar record taken from a helicopter flying over the iceberg. Horizontal axis is aircraft position; vertical axis is radar echo delay time. Aircraft flew approximately southward during this run.

iceberg entered the iceberg through its flat upper surface than through its sharply peaked top. The hyperbolic returns contain information about the subsurface shape of the iceberg, and this might be better delineated by suitable processing of the data^{4,5}.

An estimate of the attenuation of the radar pulse within the ice was made by comparing the amplitude of the iceberg bottom echo with the amplitude of reflections from the calm sea surface, assuming a normally incident signal on plane interfaces. This assumption results in an upper estimate for attenuation. Using this approach, the attenuation rate (exclusive of spherical spreading) was estimated to be $0.20 \pm 0.02 \text{ dB m}^{-1}$, implying the possibility of sounding icebergs several hundred metres thick. The attenuation rate could easily be an order of magnitude lower, which would be more consistent with previous work on polar ice with few impurities^{2,3,6}. The chlorinity of ice samples taken near the surface of the iceberg was $1-10 \times 10^{-5}$, indicating little contamination of the ice by sea water. No evidence was

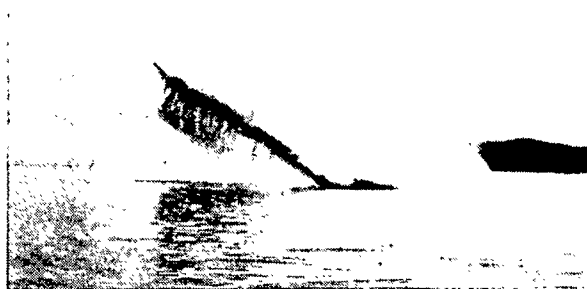
Fig. 2 Interpretation, obtained from side-scan sonographs, of iceberg underwater profile, for each of four faces.



seen for random internal scattering due to inhomogeneities, common to radar sounding measurements of temperate glaciers⁷.

Side-scan sonar has been used previously to profile icebergs⁸, by lowering a sonar transducer vertically, at a known rate, from the side of a boat. The sonar system used was built by Klein Associates, and was modified for vertical operation. A model 8A-350 acoustic transceiver, which operates at 100 kHz, and a model 402 towfish, which transmits a $1.75^\circ \times 20^\circ$ fan-shaped beam, were used.

Fig. 3 Iceberg above-water form as it rolled, viewed from the south-west. Lower photograph shows approximate final position of the iceberg during the measurements. This sequence spanned about 3 min.



As the towfish was lowered over the side of a boat, about 10 m away from the iceberg, the sonograph was displayed on a graphic recorder, and manually marked with the length of cable played out. A strong echo was received from the side of the iceberg, and the point at which this echo disappeared on the sonograph record was interpreted as indicating the depth at which the towfish had dropped beneath the lowest point of the iceberg. Interpreted iceberg profiles were made at four faces of the iceberg (Fig. 2). The overall draft of the iceberg was determined to be 18.0 ± 1.5 m.

Although the subsurface form of an iceberg can be obtained using side-looking sonar, this approach has some serious limitations. The technique is operationally very slow, and requires expensive logistic support, including a vessel. It also requires corrections for the tilt angle of the transducer from vertical and for the incident beam reaching grazing angle at the bottom of the iceberg, both of which are often difficult to estimate.

An iceberg's underwater shape clearly affects its stability, with most of an iceberg's ablation occurring beneath the waterline. As the iceberg melts it can become metastable so that only a slight perturbation is needed to initiate rolling. The wake from a small boat caused the iceberg under study to roll through 90° (Fig. 3). The underwater shape and the rolling process are important unknowns in understanding iceberg deterioration.

Although the measurements reported here are for a single, small iceberg, airborne radar shows considerable promise as a quick, safe, and accurate means of sounding icebergs. Interpretation of the radar return may be complicated by reflections from many facets within the iceberg; hence, the technique might be easier to apply to larger icebergs than to smaller ones. It might be most useful in relating the above-water form of icebergs to their subsurface draft on a statistical basis.

We thank T. Ridings, J. Johnstone, W. Winsor and J. Skidmore for assistance; A. Allan for encouragement. We also thank Memorial University Faculty of Engineering for the use of the side-scan sonar system.

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Received 1 August; accepted 28 October 1977.

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Slave–Churchill collision tectonics

THE analogue model^{1–4} of plane indentation⁵ is applied qualitatively here to the proposed collision⁶ of the Slave craton (last major deformation ~ 2,500 Myr ago) and the western Churchill craton (last major deformation ~ 1,740 Myr ago) of the Canadian Shield. The model successfully predicts several large-scale deformational features of the collision zone. McKenzie¹ has drawn an analogy between the collision of the African plate (Arabian shield) with the Eurasian plate and the impact of a hard die on a soft piece of metal. He concluded that when salients

of an approaching continent meet an Andean-type margin of another continent, further lithospheric consumption is not possible due to the buoyancy of continental crust^{1,7} and either the crust of the underthrust continent is thickened, or two or more small plates (such as the Turkish, Aegean and Iranian plates) are formed and are pushed aside to permit further convergence, or both processes occur¹.

Molnar and Tapponnier^{2–4} have presented a similar analogy to explain certain tectonic features of Asia. They believe that the collision of India with Asia is responsible for the tectonic pattern we now see in Asia. Some crustal shortening is attributed to overthrusting in the Himalayas and in Tibet but the greatest amount of shortening is attributed to lateral movement of crustal blocks out of the path of the advancing subcontinent. In their model, India is analogous to a rigid indenter, Asia to a rigid-plastic solid, and large-scale strike-slip faults in Asia correspond to slip lines formed in response to plane indentation. They appealed to differently shaped rigid dies (Fig. 1a, b) in various parts of the collision zone to explain different features of the tectonic pattern of Asia. Of particular interest is the analogy they drew between the boundary of India in the western Himalayas and in Pakistan (Fig. 2a) and a wedge-shaped indenter (Fig. 1b).

A similar analogue model can be used to predict the behaviour of the Slave craton and the type of deformation that the Churchill craton underwent during collision. Here the Slave corresponds to a wedge-shaped rigid indenter, and the underthrust Churchill to a rigid-plastic medium (Figs 1b, 2b). The model predicts at least six major deformational features of the collision zone. These are now examined in turn to determine the success of the model.

According to the model (Fig. 1b) the northern and southern common boundaries of the Slave wedge and Churchill craton should be sinistral and dextral strike-slip faults respectively. The Bathurst and McDonald faults (Fig. 2b), which form the boundaries of the Slave wedge are major sinistral and dextral strike-slip fault systems that extend for hundreds of kilometres. They can be compared with the Quetta–Chaman and Karakoram faults in India (Fig. 2a). The model further predicts that the Slave wedge should behave as a rigid body relative to the Churchill. Hoffman *et al.*⁸ consider that relatively undeformed Archean rocks of the Slave province extend as far south as a wide belt of intensely lineated mylonites that occurs south of the McDonald fault in the Churchill province (Fig. 2b). Because the Slave granites are relatively undeformed and because the mylonites are apparently cut off by the McDonald fault, Hoffman *et al.* regard the mylonites as older than the fault and not genetically related to it. The present model, however, provides an alternative explanation for these relationships in terms of the different responses of the cratons to indentation; the rigid wedge (Slave) suffers relatively little deformation whereas the rigid-plastic medium (Churchill) is deformed intensely by widespread shearing and cataclasis over the areas indicated by the slip lines in Fig. 1b.

As indentation proceeds, crustal shortening is accomplished by local crustal thickening near the apex of the wedge, by sideways motion of crustal blocks (microplates) along strike-slip faults, or by both processes. The boundary between the Slave and Churchill provinces in the apical region is the Thelon metamorphic front⁹. Belts of cataclastic gneiss and mylonite occur in a wide zone roughly parallel to the front within the Churchill province¹⁰ and much of the eastern margin of the Slave is an extensive zone of shearing¹¹. The front is bounded on the Slave side by a regional negative gravity anomaly that is truncated by the Bathurst and McDonald faults and on the Churchill side by a coextensive positive anomaly (Fig. 3a). These paired anomalies have been interpreted as an edge-effect between juxtaposed crustal blocks of different mean density and thickness⁶; the Slave block is regarded as 'normal' crust whereas the Churchill crust is locally thickened and has a somewhat higher than normal density (Fig. 3b). Gibb and Thomas⁶ have interpreted the density discontinuity separating the blocks as a cryptic suture between collided cratons. The apical region of the Slave wedge is thus a wide zone of cataclasis where underthrusting of the Churchill has been inhibited by

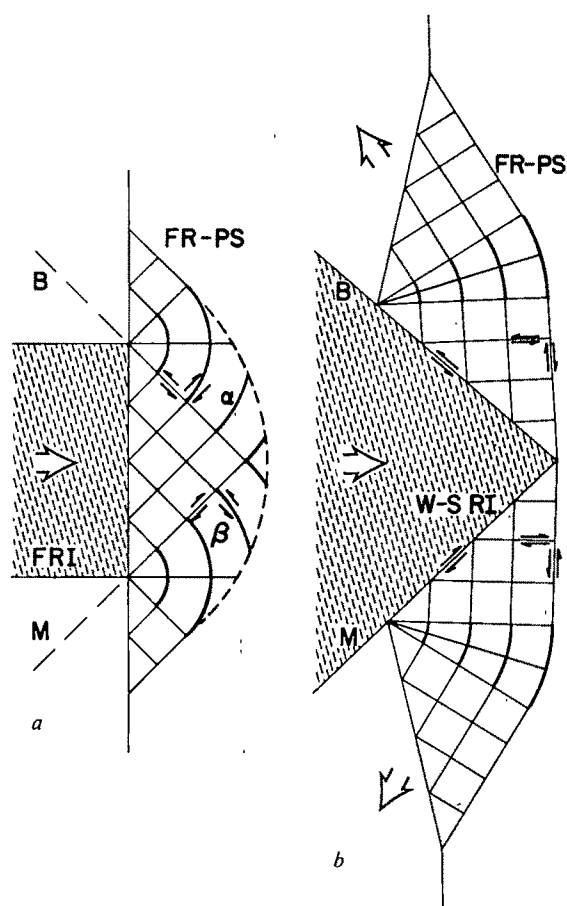


Fig. 1 Pattern of slip lines formed in the indentation of a semi-infinite flat rigid-plastic solid (FR-PS) by (a) a flat rigid indenter (FRI) and (b) a wedge-shaped rigid indenter (W-SRI) (modified from refs 1, 3, 5). Small arrows show sense of shear along α and β slip lines. Large arrows indicate direction of motion of indenters and displaced material of rigid-plastic solid. The analogous relationship of the Bathurst (B) and McDonald (M) faults to the models is also indicated.

buoyancy of continental crust resulting in localised crustal thickening as predicted by the model. It can be compared with the Pamir thrust belt in India (Fig. 2a).

Within the Churchill province strike-slip faults bounding microplates that may have developed north and south of the Slave wedge should display dextral and sinistral motion respectively (Figs 1b, 2b). Although the geology of these critical areas is relatively poorly known, a well-developed, unnamed, major fault mapped as a wide mylonite zone¹² (Fig. 2b) along with the Bathurst fault may define the boundaries of a northern microplate pushed sideways from the path of the advancing Slave wedge until such time as suturing was completed. A linear, negative magnetic anomaly coincides with the mylonite zone and extends north towards the coast and south to the Bathurst fault system with a total length of almost 300 km, confirming that the extent of the fault is much greater than shown on maps (Fig. 2b). The model predicts that the mylonite zone should be a dextral transcurrent fault (Fig. 1b) with a role similar to that of the Herat fault in Asia (Fig. 2a). The extension of the McDonald fault to the north-east (Fig. 2b) may be compared with the Talasso-Fergana fault in Asia (Fig. 2a) which has the same position relative to the wedge and the same sense of shearing. The proposed microplate south of the McDonald fault may be delimited to the east by either the sinistral Black Lake or Allan faults (Fig. 2b) and their northerly extensions or perhaps by an as yet unidentified fault corresponding to the Altn Tagh fault in Asia (Fig. 2a). The Black Bay fault is another possible boundary fault; its geometry is consistent with the slip line pattern but its sense of movement is dextral.

North of the Bathurst fault reconnaissance mapping¹² has revealed several mylonite zones but not in sufficient detail to determine the strike-slip fault pattern predicted by slip line field theory (Fig. 1b). In the apical region, however, the effects of indentation are graphically illustrated by faults which dissect the Thelon magnetic high¹³ (Fig. 3c). Here major splays of the Bathurst¹³ and McDonald¹⁴ fault systems correspond to the pattern of slip lines formed by indentation of a flat die (Fig. 1a). Splay faults associated with the Bathurst fault system cut the magnetic high sinistrally¹³ as predicted by theory (β slip lines of Fig. 1a). A second set of faults almost normal to the first corresponds to α slip lines with a dextral sense of movement (Fig. 1a). South of the McDonald fault, the whole area as far south as Lake Athabasca is characterised by complex fault patterns¹⁵ which include wide mylonite zones, anastomosing faults related to the McDonald fault¹⁶ and to other major transcurrent faults, normal faults like those bordering the Nonacho basin¹⁷ and regional thrusts.

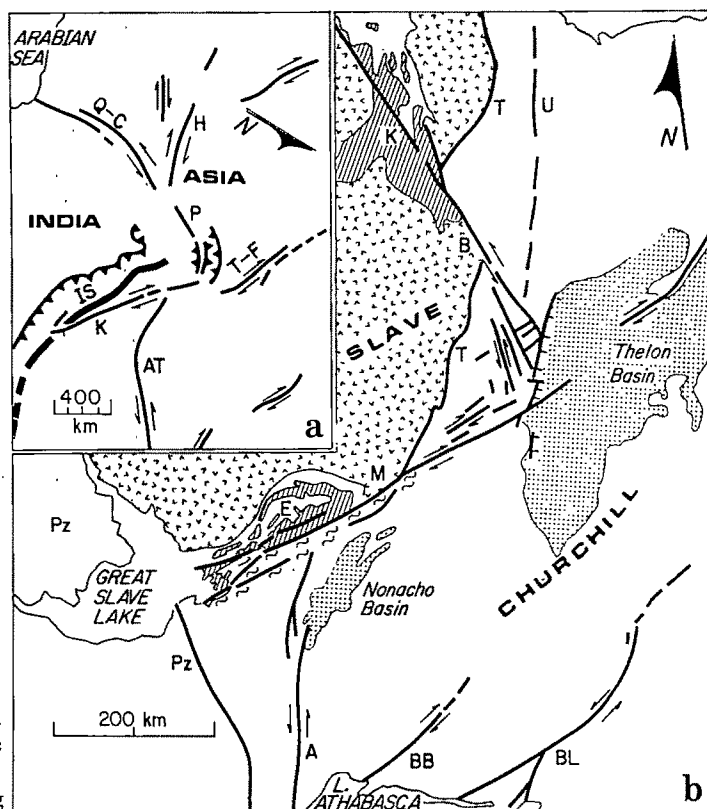


Fig. 2 Comparison of the major tectonic elements of (a) India and Asia in the vicinity of the Pamirs (P) (after ref. 3) with those of (b) the Slave-Churchill boundary zone in Canada (modified in part from refs 12-18). a, Q-C, Quetta-Chaman fault; H, Herat fault; K, Karakoram fault; T-F, Talasso-Fergana fault; AT, Altn Tagh fault; IS, Indus suture. b, B, Bathurst fault; M, McDonald fault; T, Thelon front; U, unnamed mylonite zone and its extensions to north and south; A, Allan fault; BB, Black Bay fault; BL, Black Lake fault; K, Kilohigok basin; E, East Arm foldbelt. Ornament, v pattern, Slave province; waves, mylonite zone; diagonals, Apehian basins; dots, Helikian basins; Pz, Paleozoic cover.

According to the wedge indentation model, rocks of the ancient Churchill continental margin originally at the apical contact region should be squeezed out and transported along the bounding faults of the indenter. Exotic breccias that have proved difficult to explain occur in the East Arm foldbelt in several fault-controlled zones associated with the McDonald fault¹⁸. Breccia fragments include both Apehian sediments and Archean granite and range in size from large blocks tens of metres in length, to 'normal' breccia containing fragments a few centimetres in length set in a finer matrix. A possible explanation for these exotic breccias and other allochthonous fault-bounded blocks in the foldbelt is that they are chaotic fragments of the ancient Churchill

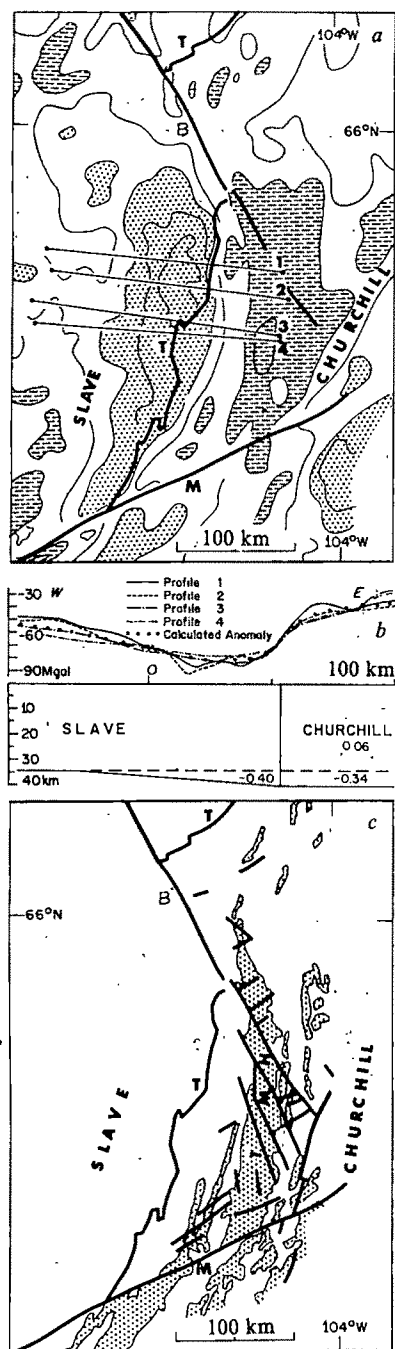


Fig. 3 a, Bouguer anomaly map of Slave-Churchill boundary zone (modified from ref. 6). Dots, anomalies < -60 Mgal; dashes, anomalies > -40 Mgal; 1-4, locations of profiles shown in b; B, M, T as in Fig. 2b. b, Profiles 1-4 showing Bouguer anomalies, calculated anomaly and interpreted crustal model (modified from ref. 6). Density contrasts in g cm^{-3} . c, Schematic magnetic map illustrating offsets of the Thelon magnetic high (TMH) by sinistral and dextral faults in the Slave-Churchill boundary zone (modified from ref. 13). Dots, total field magnetic anomalies $> 61,000$ nT; B, M, T as in Fig. 2b.

margin and craton that have been transported to their present locations in the Slave-Churchill boundary fault zone during the process of collision (indentation). Similar unexplained exotic breccias occur within the Aphebian Goulbourn Group rocks of the Kilohigok basin in the Bathurst fault zone¹⁹ and may have the same origin. Nappes have been described recently by Hoffman *et al.*⁸ in the East Arm foldbelt. They are up to 70 km long and 10 km wide⁸ and may have been ruptured and deposited during the same process; alternatively they may have been deposited by obduction of basin fill from the south during closure of the diminishing sea between the Slave wedge and Churchill craton. The presence of

compressional structures in the East Arm foldbelt and the juxtaposition, on either side of the foldbelt, of crustal blocks of distinctly different metamorphic history, metamorphic age, and deformational history tend to support a collision model rather than the aulacogen (failed rift) model proposed by Hoffman²⁰ in which tensional features should predominate and bordering cratons are likely to be similar.

These preliminary results suggest that the wedge indentation model or other variants of the indentation model may be useful for predicting deformational features of other proposed collision zones within the Canadian Shield²¹ as originally suggested by Molnar and Tapponnier².

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Received 5 October; accepted 3 November 1977.

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Inbreeding and dispersal in the great tit

IN populations which normally outbreed, matings between close relatives can result in a decrease in the viability and fertility of their offspring. Such inbreeding depression has been shown in a number of laboratory studies of insects¹, birds² and mammals³. We present here the first detailed evidence of inbreeding depression in a natural population and support for the hypothesis⁴ that one function of dispersal between birth and breeding sites is to reduce an individual's chance of inbreeding.

The paucity of long term studies on known individuals has meant that the frequency and consequences of inbreeding in natural populations are virtually unknown. Inbreeding has been reported in the great tit (*Parus major*)^{5,6}, the yellow-eyed penguin (*Megadyptes antipodes*)⁷, the song sparrow (*Melospiza melodia*)⁸, the deer mouse (*Peromyscus maniculatus*)⁹, in addition to probable cases in the gorilla (*Gorilla gorilla*)¹⁰ and olive baboon (*Papio anubis*)¹¹. In all cases, except the great tit⁶, no estimate was made of the relative frequency of inbreeding and the extent of inbreeding depression. Bulmer⁶ made an initial investigation of inbreeding using the great tit data collected from 1964-70 in Wytham Wood near Oxford and we have made a more extensive analysis, using the data collected in the period 1964-75.

From 1964 to 1975 there were 885 pairings in which the identities of both the male and the female were known. Pairs are formed both from birds born in the wood (residents) and from those born outside (immigrants). Details of the study area can be found elsewhere^{12,13}. The 885 pairs consisted of 194 matings between resident males and resident females, 239 between resident males and immigrant females, 158 between immigrant males and resident females and 294 between immigrant males and immigrant females. Of the total number, 13 (1.5%) were inbreeding pairs: five mother-son, seven

brother-sister (each pair from siblings in the same brood) and one aunt-nephew. If we include the same pairs breeding in 2 or more years, the total number of matings was 1,000, of which 16 (1.6%) were inbred. Two mother-son and one brother-sister pair each bred twice. The coefficient of inbreeding is

$$F = [(5 \times 0.25) + (7 \times 0.25) + (1 \times 0.125)] / 885 = 0.0035$$

It is probable that all cases of inbreeding between close relatives have been found⁶. The incompleteness of some of the pedigrees may have meant that a small, but undetected, number of inbreedings may also have occurred between more distant relatives, but such cases would produce only a slight increase in the coefficient of inbreeding.

From figures presented by Richdale⁷, we have also calculated the coefficient of inbreeding in the yellow-eyed penguin. In 244 pairings which produced 490 matings he found three brother-sister, one half brother-half sister and three second cousin cases of inbreeding. The coefficient of inbreeding in this instance is

$$F = [(3 \times 0.25) + (1 \times 0.125) + (3 \times 0.0156)] / 244 = 0.0037$$

The frequency of close inbreeding (coefficient of kinship ≥ 0.125 (ref. 14)) is 1.6%. The figures are close to those found in the great tit: both species exhibit low levels of dispersal.

In laboratory studies the timing of reproduction, litter size, infant or fledging mortality^{2,3} and offspring viability and fertility^{3,15} may all be affected adversely by inbreeding. The laying date, clutch size, fledging success and number of offspring subsequently recorded breeding in the wood for all matings between relatives in the great tit are shown in Table 1. We have also calculated the dispersal distance from natal to breeding sites by all males, and those females mated to their brothers. In the cases of mothers and aunt mating with sons and nephew respectively, the appropriate measure of dispersal was the distance from previous outbreeding site to the subsequent inbreeding locality. For all these measures we have calculated the expected values from comparable outbred matings in which both parents were known (all second broods and relays have been excluded). Since there are yearly and age variations in laying date, clutch size¹² and dispersal (our work in preparation),

expected values are for each age-year category in question. We have only distinguished between first year and adult age classes, since sample sizes would otherwise have been small. The expected values for nesting success and the number subsequently recorded breeding in the wood have been calculated from the whole period 1964-75. We have excluded from the analysis all cases, in both inbreeding and outbreeding pairs, where breeding success was affected by predation, disturbance or experimental manipulation of brood size. For example, a great spotted woodpecker (*Dendrocopos major*) drilled a hole in the side of a nest box used by a mother-son pair in 1969. The incident occurred at hatching time and no offspring subsequently fledged; this pair has not been included in the calculation of nestling mortality.

On the three occasions when related birds bred in more than one year we have assumed that variables, such as laying date, are not independent measures. Consequently, in the analysis, we have averaged the observed and expected values for the 2 years.

The coefficient of kinship between mother and son is 0.25, as is, on average, that between full sibs, whereas that between aunt and nephew is 0.125. In our analysis we have included the latter example even though inbreeding depression would be expected to be less severe.

There is no evidence that inbreeding pairs produce smaller clutches than outbreeders (Wilcoxon test, $T = 32$, $n = 13$). Unexpectedly, however, the females mated with close relatives commenced laying before other females ($T = 15$, $n = 13$, $P < 0.05$). The reason for earlier laying is unknown, but may be connected with dispersal distance (our work in preparation). The result differs substantially from the laboratory study of *Peromyscus maniculatus*, in which siblings induced to mate, showed a delayed onset of reproduction when compared with unrelated pairs³.

Nestling mortality is higher among inbreeding than among outbreeding pairs ($T = 23$, $n = 15$, $P < 0.05$). We cannot distinguish between eggs which failed to hatch and offspring which died after hatching but before fledging. We refer collectively to the mortality as nestling mortality, which was, on average, 27.7% among inbreeders, whereas the expected mortality from equivalent sized clutches from outbreeders would be 16.2%.

Of the 86 inbred offspring which successfully fledged from mother-son and full sibling matings, five (5.8%) were subse-

Table 1 Observed and expected values for inbreeding pairs of great tits, 1964-75

Year	♂ Distance dispersed (m)		♀ Distance dispersed (m)		Day of first egg		No. of eggs		No. of fledglings		No. recovered breeding	
	O	E	O	E	O	E	O	E	O	E	O	E
Mother-son												
1965	14	466.7	0	77.7	122	126.9	8	8.38	8	6.72	0	0.84
1967	—	—	—	—	129	121.5	7	8.10	4	5.97	0	0.36
1968	120	382.7	50	90.5	115	120.1	6	8.35	3	4.83	1	0.18
1969*	—	—	—	—	133	123.2	7	8.28	(0)	—	—	—
1971	91	623.9	110	91.7	124	126.9	8	8.41	3	6.72	0	0.18
1973	110	453.1	105	55.9	121	123.8	9	8.48	8	7.53	0	0.84
1974	0	378.5	0	64.0	132	131.8	10	7.06	6	8.43	0	0.73
Brother-sister												
1966	151	690.3	151	826.4	127	133.1	10	8.29	6	8.43	0	0.73
1967	500	340.1	500	912.5	118	121.5	10	8.10	6	8.43	0	0.73
1968	—	—	—	—	116	120.1	10	8.35	9	8.43	0	0.79
1970	237	463.1	237	944.7	123	126.8	10	9.83	9	8.43	0	0.79
1971	970	623.9	970	749.7	131	126.9	7	8.41	4	5.97	1	0.36
1972	1,486	453.1	1,486	758.2	126	133.1	9	8.29	6	7.53	1	0.73
1972	632	623.9	632	749.7	119	122.4	8	8.03	7	6.72	2	0.65
1972	356	623.9	356	749.7	116	122.4	9	8.03	7	7.53	0	0.65
1977	835	—	835	—	—	—	6	—	6	4.83	—	—
1977	380	—	380	—	—	—	8	—	6	6.72	—	—
Aunt-nephew												
1966	41	340.1	36	90.6	128	133.1	9	8.29	8	7.53	1	0.84

Observed (O) and expected values (E) for inbreeding pairs 1964-75. Pairs breeding in more than 1 yr are bracketed together. Two brother-sister pairs recorded in 1977 were used only in the analysis of nestling mortality.

* Breeding disturbed by woodpecker at time of hatching.

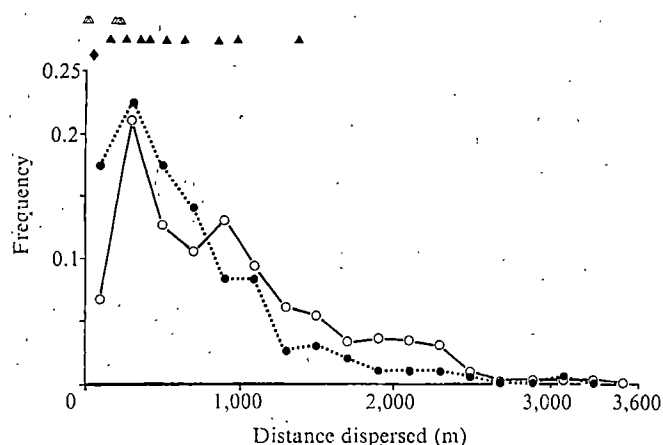


Fig. 1 Distance dispersed by outbreeding individuals between natal site and site of first breeding. The distance localities of inbreeding are marked for males only, when mated to mothers and aunt, and for both males and females, moving the same distance, in brother-sister pairs. Dispersal: , males; ————, females. Inbreeding: Δ , mother-son; \blacktriangle , brother-sister; \blacklozenge , aunt-nephew.

quently recovered breeding in the wood. If we include the one produced by the aunt-nephew pair, six (6.4%) returned from 94 offspring. The expected number from normal broods were respectively 8.56 (10.0%) and 9.40 (10.0%). If we assume that the number of recoveries fits a Poisson distribution, then the probabilities of obtaining five or less from 86 and six or less from 94 fledglings are 0.145 and 0.174 respectively. Mortality after fledging is not significantly greater than expected.

The six inbred offspring (three males and three females) subsequently outbred and produced an average clutch of eight eggs. The nestling mortality of their offspring was 16.7%. None of the offspring of mother-son or brother-sister pairs survived longer than one breeding season, whereas that of the aunt-nephew pair survived 2 years.

The distance dispersed between natal and breeding site may affect the chances of mating with a close relative. The patterns of dispersal of normal juvenile males and females, together with the locations of inbreeding are shown in Fig. 1. Females disperse further than males in their first year⁶; but subsequently, there is little movement and individuals tend to retain the same territory from one year to the next (our work in preparation). These patterns of dispersal lead to the expectation that sons mating with their mothers would have dispersed substantially less than expected. In sibling matings, brothers should move more than the expected median distance or sisters less than expected, or both. These predictions are supported by the data, although formal testing is inappropriate because of the lack of independence of dispersal between related inbreeding individuals. On average, sons mated with their mothers moved only 67 m from natal to breeding site, 85% less than the expected distance (461 m). Males mated to their sisters dispersed 13.5% more than expected, 619 m as opposed to 545 m, whereas sisters moved less than expected (24%), 619 m rather than 812 m.

From the distribution of dispersals it is possible to calculate the expected number of inbreedings from the model produced by Bulmer⁶. For close relatives these are: mother-son, 6.2 (observed 5); brother-sister, 7.35 (observed 7); and father-daughter, 2.2 (observed 0). More examples are necessary, before it is possible to estimate whether fewer inbreedings are occurring than would be expected.

We have shown that inbreeding pairs have a lower breeding success, resulting from higher nesting mortality, than normal pairs and that the chances of inbreeding vary with dispersal distance. Since the avoidance of such matings is one factor influencing dispersal, it is possible that the asymmetry in movement between males and females in many sedentary species

is maintained by the detrimental effects of inbreeding^{1,16-18}.

We are grateful to Tony Martin and Peter Croydon for computer programming support. We thank Michael Bulmer, Tim Clutton-Brock and members of the Population Biology Group at Sussex for comments, and John Maynard Smith for much help and encouragement. This work was supported by the SRC.

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Received 13 October; accepted 9 November 1977.

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Cortical magnification factor predicts the photopic contrast sensitivity of peripheral vision

THE area of visual cortex devoted to the analysis of a constant-size region in the visual field diminishes progressively for more peripheral locations. The change is described quantitatively by the cortical magnification factor, which indicates the linear extent of cortex in mm corresponding to one degree of visual angle at various eccentricities (angular distances from the middle fovea). The human cortical magnification factor has been estimated by Cowey and Rolls¹ from the data of Brindley and Lewin², who mapped the phosphenes (sensations of light) caused in the lower nasal visual field by electrical stimulation of the human visual cortex. Building on these results, we have studied the spatial contrast sensitivity functions in man at various eccentricities. We used two methods: in one the retinal image sizes of the test gratings were kept similar at different eccentricities and in the other, the calculated sizes of cortical projections of the test gratings were made similar at different locations. Our results indicate that peripheral contrast sensitivity and acuity are inferior to foveal performance, because of the diminished cortical projection area.

Four experienced subjects fixated a small point of light binocularly in a dark room; a bite board aided steady fixation. The contrast sensitivity at various eccentricities along the nasal horizontal and inferior vertical visual-field meridians of the right eye was measured by determining the inverse of the threshold contrast for each of a series of sinusoidal gratings. The gratings were generated on a white cathode-ray screen³, masked to expose only half of a round field, 16 cm in original diameter (see inset, Fig. 1a). The screen was perpendicular to the visual axis if viewed directly and at the same distance as the fixation point; the screen was not visible to the left eye.

The average luminance of the screen was continually

11 cd m^{-2} and the diameter of the natural pupil varied from 6 to 7 mm. (No artificial pupil was used because it limits the visual field.) The photopic average retinal illuminance was thus about 365 Trol (1 troland = $1 \text{ cd m}^{-2} \times \text{pupillary area in mm}^2$). On our display this corresponds to about 1,030 scotopic Trol, indicating a photopic adaptation level.

Thresholds were determined by using a computer-controlled forced-choice method: the subject received two sound signals and had to decide during which signal a grating was presented. Another sound indicated the wrong choices. After each block of four consecutive correct responses, contrast was decreased by 2 dB and every wrong choice led to a contrast increment of 2 dB: the contrast required for a probability of 0.84 of correct response was estimated from the turning points as described by Wetherill and Levitt⁴. The critical stimulus to be detected was a stationary vertical grating with a non-zero contrast for 0.5 s; the control stimulus was otherwise identical but had a zero contrast. To avoid the bias caused by the Troxler effect (fading of stimuli in peripheral vision) the subject initiated each threshold trial only when the display was clearly visible. Control experiments showed that results essentially similar to those of the detection method are obtained from a two-choice recognition of grating orientation or direction of movement.

Figure 1 shows the results for subject V.V. in the lower half of the vertical meridian; the results of other subjects and of the nasal horizontal visual field were similar. In the experiments of Fig. 1a the display was viewed at a constant distance of 458 cm. The retinal images of the gratings subtended $1 \times 2^\circ$. At increasing eccentricities, contrast sensitivity and visual acuity (assessed from the resolution of high-contrast gratings) decreased as similar decelerating functions of eccentricity.

Figure 1b presents results for the same eccentricities, but in this case both the spatial frequency and the size of the gratings were scaled by the inverse of the cortical magnification factor estimated from the points calculated by Cowey and Rolls¹. Thus, the cortical topographical representations of the gratings became equal in size at various eccentricities. For the series of eccentricities: 0, 1.5, 4, 7.5, 14 and 30° the following magnification factors were assumed: 7.75,

5.25, 3.44, 2.31, 1.24 and 0.49 mm. The scaling of gratings by the inverses of these values was accomplished by keeping the gratings constant and moving the subject closer to the display; the effects of the changes of accommodation on acuity were partially compensated by corrective lenses. The viewing distances for the semicircular gratings with radius 8 cm were 458, 312, 204, 137, 73.3 and 28.3 cm. Thus the gratings subtended $1 \times 2^\circ$ at $E=0$, and $15.8 \times 31.6^\circ$ at $E=30^\circ$, with corresponding intermediate retinal sizes. Assuming that the magnification factors were correct, the crescent-shaped cortical projection fields had a constant size of about $7.75 \times 15.5 \text{ mm}$ and the fields stimulated at neighbouring eccentricities were tangential to each other.

After scaling, the widely separate contrast sensitivity functions of different eccentricities collapsed to an apparently single function, as in Fig. 1b. This is surprising, because different types of retinal ganglion cells may dominate in peripheral and foveal visual systems, and the projections reach several locations in the brain⁵. A good correlation between conventional measures of visual acuity and the magnification factor^{1,6} can be explained by postulating a homogeneous detection system subserving acuity, but there is evidence that different types of mechanisms mediate the detection of low and high retinal spatial frequencies⁷.

A minor deviation from the unity of the scaled functions occurred at high spatial frequencies (Fig. 1c), but this was expected for optical reasons. The optical limitations of contrast transfer with a pupil size of 6–7 mm are considerable at high retinal spatial frequencies, but they should not affect the transfer of low retinal spatial frequencies⁸ that represent high 'cortical' spatial frequencies in peripheral vision. Figure 1c shows that sensitivity to the highest 'cortical' spatial frequencies improves somewhat at greater eccentricities, as would be expected if optical factors limited performance at small eccentricities. The small overall decrease of sensitivity from $E=0^\circ$ to $E=1.5^\circ$ reflects the Troxler effect and also our uncertainty regarding the exact value of the foveal magnification factor.

We conclude that the foveal contrast sensitivity function can be generalised for any part of the visual field when two structurally determined adjustments are made: one is based

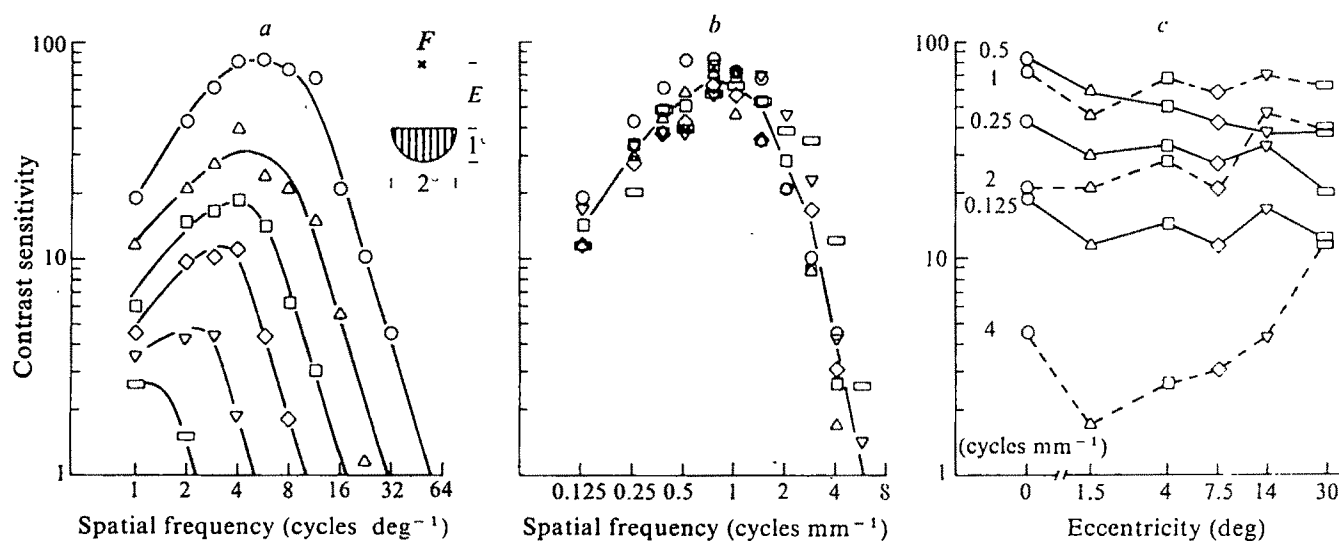


Fig. 1 Contrast sensitivity as a function of spatial frequency and eccentricity (E) in the lower visual field. *a*, The retinal images of various sinusoidal test gratings were similar at different eccentricities as shown in the inset. Spatial frequency indicates the bar density of gratings in cycles per degree of visual angle and eccentricity refers to the angular distance between the fixation point (F) and the nearest edge of the semicircular vertical grating. Curves represent values of E as follows: \circ , 0° ; \triangle , 1.5° ; \square , 4° ; \diamond , 7.5° ; ∇ , 14° ; \blacksquare , 30° . *b*, Cortical projections of the test gratings were similar at different locations. The symbols are as in *a* regarding eccentricity in the visual field. Spatial frequency indicates the estimated bar density in cycles per mm on the primary visual cortex. *c*, Part of the data of *b* replotted to reveal the expected improvement of sensitivity as a function of E at high cortical spatial frequencies.

on the cortical magnification factor and the other on the modulation transfer function of the eye optics. The result suggests that, using suitable temporal conditions, a picture can be made equally visible at any eccentricity by scaling its size by the magnification factor, because the contrast sensitivity function represents the spatial modulation transfer function of the visual system for near-threshold contrasts. The results by Anstis⁹ on the recognition of letters in peripheral vision agree with this generalisation.

The invariance of the photopic contrast sensitivity functions of different but equally large regions of the visual cortex indicates a striking functional homogeneity of image processing machinery at central levels of the visual system. This functional homogeneity is not normally evident because it is masked by various early transformations of the signals entering the cortex.

We thank the Finnish Academy and the Finnish National Fund for Research and Development for support to V.V., and Pekka K. Lehtio and Pentti Laurinen for design of apparatus and programmes.

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Is *Acanthaster planci* an *r*-strategist?

THE debate over the cause of plagues of the crown-of-thorns starfish, *Acanthaster planci*, has been as long as the plagues themselves (for reviews see refs 1-3). Most recently, two lines of reasoning based on ecological concepts have been used to suggest that the causes of the population explosions should be sought in some abnormal event, and thus man has been implicated²⁻⁴. I believe that a more detailed consideration of the ecology of *A. planci* does not support this view; rather, it suggests violent population fluctuations without the assistance of man's activities.

It has been predicted² that complex ecosystems such as coral reefs should be resistant to perturbations because of the supposed stabilising effect of high trophic complexity². It is now more widely thought⁶⁻⁸ that the stability often apparent is a consequence of a highly predictable environment, which, it is believed, on coral reefs² allows a great diversity of organisms to specialise on the large number of spatial and biotic niches created there. Such specialised species are characterised by small, stable population sizes and low reproductive rates, and are long-lived.

Grassale's descriptive framework⁷ coincides with Pianka's visualisation of an *r*-*K* continuum⁸, the two extremes representing opposing bionomic strategies for survival in the face of natural selection: *r*-strategist species (or 'opportunists'^{7,10}) tend to exploit unpredictable and/or

short-lived habitats¹¹ by devoting energy and resources to maximising reproductive potential; *K*-selected species (or 'specialists'⁷) exhibit conservative strategies for survival in predictable environments, and are adapted against pressures from predators and inter-specific competition.

Examining some of the important sessile components of the coral reef ecosystem within this framework, it seems that most corals¹² and sponges¹³, and several other organisms¹⁴ exhibit conservative strategies in view of their massive size, long life, and adaptations for inter-specific competition, although some¹⁵ are adapted to exploiting unpredictable habitats.

Where does *A. planci* fit into this picture? Its bionomic strategy is indicated by features of its ecology and life history⁹ which are shown in Table 1. There are several other coral reef echinoderms whose population distributions, like that of *A. planci*, are patchy but locally abundant, and they have also been examined within this framework, with the limited data available.

A. planci has probably a greater range within the coral reef environment than any other echinoderm, being found virtually wherever there is coral. Most other echinoderms are restricted by their food requirements to narrow zones. Studies in the Red Sea³ have indicated some density-dependent predation by fish, but it seems that when population densities become very high, as on the Great Barrier Reef², they escape the control of predators; migration then becomes important. Population sizes vary markedly in time and place¹⁻³; normal population densities are rather low, and 'dense populations' are uncommon¹⁶. Dense populations can persist aided by the virtual absence of inter-specific competition for coral prey from other reef inhabitants: most fish or other coral predators only graze polyps sub-lethally or kill small parts of colonies^{17,18}. None, except perhaps donkey-fish (*Bulbometopon muricatus*) locally (my observations), would affect significantly the food available to *A. planci*: it is apparently exploiting what is virtually an ecological vacuum.

Whereas coral is killed by *A. planci* and takes some years to be replaced, the nature of the food resources of many other common coral reef echinoderms (for example, the starfish *Linckia multiflora* (my observations) and *L. laevigata*¹⁹—substrate feeders, primarily on coralline algae, or the holothuroid *Holothuria atra*—a sediment feeder²⁰) is such that it is continuously being renewed: in their more restricted zones, they compete intra- and inter-specifically for food, near the carrying capacity of their environment. Thus, although the potential for rapid increase in population numbers is present in many coral reef echinoderms, by virtue of the large numbers of larvae produced, adult numbers of most species would seem to be limited by restricted zonation and food availability.

By contrast, *A. planci* feeds selectively at low densities, and when its population density increases, it adapts to unavailability of preferred species²¹, feeding on all scleractinian corals^{1,2}. Also, being relatively mobile, it may exploit extensive areas of reef when its food supply becomes locally depleted², and in these ways it overcomes the problem of intra-specific competition for food.

Table 1 shows that many of its characteristics indicate *A. planci* to be strongly *r*-selected, while other common coral-reef echinoderms exhibit more moderate strategies. But some aspects of *A. planci*'s reproductive strategy, being different from those predicted for *r*-selected terrestrial animals, seem to contradict this view. Upon examination, it becomes clear that different considerations must be applied. We may predict that *r*-selection would favour the production of large numbers of larvae, and most of the commoner species of coral reef asteroids, including *A. planci*, produce many small, planktotrophic, pelagic larvae²². Their survival, however, is probably very

Table 1 Bionomic strategy of *Acanthaster planci* and other common coral reef echinoderms as indicated by life-history characteristics

Correlates of <i>r</i> - and <i>K</i> -selection*	Characteristics of <i>A. planci</i>	Strategy indicated <i>r</i> <i>K</i>	Other common coral reef echinoderms†	Strategy indicated <i>r</i> <i>K</i>
Variability/predictability of environment	Most zones on coral reefs, 1–50 m depth	+	Predictable, restricted zonation	+
Mortality	Density-dependent at low densities, but escapes this control at higher densities; migration important	+	Not known	—
Survivorship	Extensive mortality of larvae	+	Extensive mortality of larvae	+
Population size:				
—variability in time	Variable	+	Less variable than <i>A. planci</i> ?	?
—relation to carrying capacity	Usually low	+	Often high	+
—saturation of community	Ecological vacuum	+	More saturated	+
—recolonisation	Migration of larvae important	?	Larvae migrate; asexual reproduction occurs in some species	?
Competition:				
—inter-specific	Negligible	+	Varies with species	—
—intra-specific	Low except at very high densities	+	Limits numbers locally	—
Characteristics predicted as favoured by selection:				
—rate of development	Fast	+	Relatively slow	+
— r_{max}	Potentially very high	+	Potentially very high	+
—age of sexual maturity	1–3 yr		Not known, but probably comparable	+
—body size	Large compared to other coral reef echinoderms		Varies with species, but all possess anti-predator defences	+
—reproductive strategy	Repeated		Repeated	+
Longevity	8 yr		Not known, but probably comparable	+

Data sources available from author. +, Denotes clearly indicated *r* or *K* strategy; ?, strategy not clear.

*After Pianka⁹.

†Details of individual species are omitted for sake of brevity. Actual species compared are: *Culcita novaeguineae*, *Linckia multiflora*, *L. laevigata*¹⁸, *Diadema setosum* and *Holothuria atra*¹⁹.

uncertain^{23,24}, particularly in the case of *A. planci*, whose high survival rates of larvae may result from unusual levels of water salinity and temperature and food supply (ref. 25 and R. Pearson quoted in ref. 6). The uncertainty in recruitment of juveniles must be counteracted by a shift towards *K*-selection in the adult, reflected in increased longevity, as demonstrated in some marine fishes²⁷: in *A. planci* the life span is believed to be about eight years²⁸. Anti-predator defences (which complement increased longevity) are also present in most common coral reef echinoderms; *A. planci* has spines and a cryptic behaviour, as do many echinoids, and other asteroids have tough skins or heavily-calcified skeletons²². Large size may act in the same way¹¹.

It would be unrealistic to attempt to model mathematically the population dynamics resulting from the bionomic strategy of *A. planci* as outlined above, owing to its apparently 'fugitive' nature (local populations usually have a unimodal age distribution²⁴).

Clearly *A. planci* is not a 'classical' *r*-strategist in the sense of having an unstable or short-lived habitat, but its life-history characteristics indicate a coherent strategy for exploiting what is, surprisingly, a virtual ecological vacuum. In some ways its strategy resembles that of certain insect pests, for example, the spruce budworm²⁹, which conform broadly to *r*-strategies, but exploit longer-lived habitats.

In view of the very high reproductive potential (r_{max}) and the possibly long period before environmental conditions favour massive larval survival, the large fluctuations of local populations which characterise *r*-strategists¹¹ can only be exaggerated in the case of *A. planci*. Thus it is inappropriate to regard the low population levels usually observed^{2,3,16} as 'normal' and high population levels as 'abnormal': periodic plagues are inherent to this mode of living and may well be necessary for survival of the species. It is unfortunate for many fish and other reef inhabitants that such plagues result in the destruction of their habitat³⁰—the effects are comparable to those of destroying the canopy of a tropical rain forest. Coral reefs

are also valuable to man, and it is reflection of his subjective viewpoint as a *K*-strategist, striving to maintain his resources, that the terms 'plague' and 'population explosion' tend to be imbued with emotion.

This paper arose from work carried out during the crown-of-thorns research project funded by the Ministry of Overseas Development. I thank John Lawton and Rupert Ormond for stimulating discussions.

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Received 21 March; accepted 18 October 1977.

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Requirement for cell differentiation in *Dictyostelium discoideum*

THE generation of cell diversity during the development of multicellular organisms involves a series of decisions between alternative pathways of differentiation^{1,2}. In many instances the fate of a cell depends on its position, so that pattern formation and cell differentiation can become facets of the same process³. These phenomena occur in a simple form in the slime mould *Dictyostelium discoideum* where there are only two major pathways of differentiation—one leading to stalk cells and the other to spores. On starvation the individual amoebae aggregate chemotactically to form multicellular masses. Somewhat later the aggregates can be shown to consist of two distinct cell types: prestalk cells which are localised in the anterior and prespore cells in the posterior of the migrating slug⁴. We are interested in how the formation of these cell types is controlled. One approach to this problem involves experimentally inducing differentiation of cells that are somehow prevented from completing normal development. Starving cells plated on agar with a Cellophane overlay aggregate but remain constrained as a monolayer and do not progress to stalk or spore. But addition of 1–5 mM cyclic AMP to the agar results in efficient induction of stalk cell formation provided that a high cell density is used^{5–7}. At low cell density stalk cells do not form unless a low-molecular weight

factor, released by cells at high density, is also provided⁷. We show here that although no mature spores form in conditions of high cell density giving a high yield of stalk cells large numbers of prespore cells do appear. Many of these later transform into stalk cells. Prespore cells do not arise at low density but the density requirement for prespore cell formation, unlike that of stalk cells, cannot be efficiently replaced by the low-molecular weight factor. Five mutants isolated by virtue of their ability to form mature spores under Cellophane likewise do not form spores at low cell density even in the presence of the 'helping' factor. Thus entry of developing *Dictyostelium* cells into the prespore pathway apparently requires some cell interaction different from or additional to that required for entry into the stalk pathway.

Our initial observations, made by electron microscopy, were concerned with the induction of prespore vacuoles (PSVs), which most workers consider to be specific markers of prespore cells^{8–12}. HMI cells were plated at high cell density on agar and overlaid with Cellophane as described in the legend to Fig. 1. In the absence of added cyclic AMP few PSVs and no stalk cells were observed (Fig. 1). When 5 mM cyclic AMP was included in the agar, large numbers of PSVs were induced. They first appeared by 15 h of induction, reached a peak number by 24 h and thereafter declined in numbers as mature stalk cells accumulated. The latter were never seen to contain a PSV.

The experiment described above was rather time consuming and so in the following experiments we identified prespore cells by their specific fluorescent staining with absorbed anti-spore serum^{13,14}. Figure 2b again demonstrates the dramatic induction of prespore cells by 5 mM cyclic AMP at high cell density. This result is in close qualitative agreement with that obtained by electron microscopy. Precedents for these results are to be found in the reports of the occurrence (but not the induction) of prespore cells in the presence of 1 mM cyclic AMP^{15,16}. The kinetics of prespore induction shown in Figs 1 and 2b suggest that

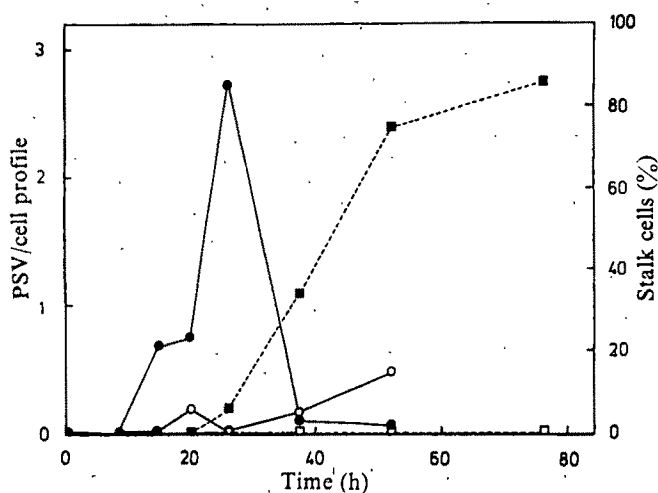
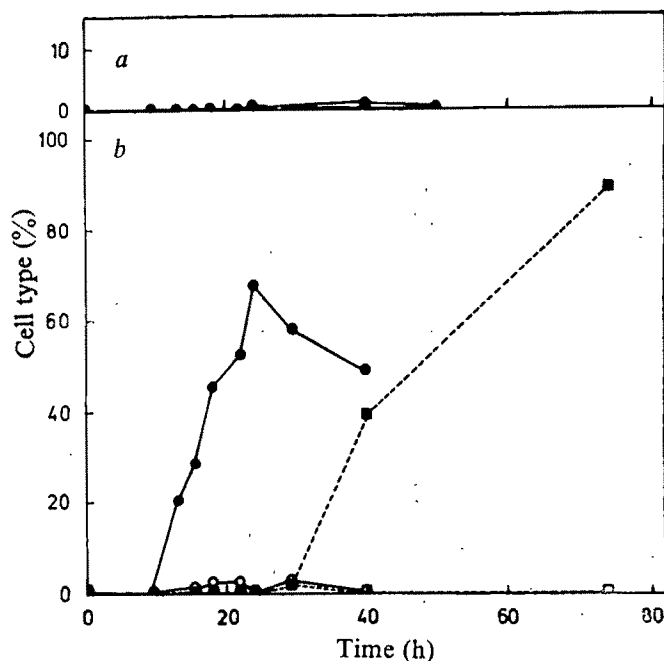


Fig. 1 Induction of prespore vacuoles and stalk cells by cyclic AMP in cells plated at high density under Cellophane. ○, Prespore vacuoles, no cyclic AMP; □, stalk cells, no cyclic AMP; ●, prespore vacuoles + cyclic AMP; ■, stalk cells + cyclic AMP. HMI cells (HMI is an acriflavin resistant strain derived from V12 M2) were grown on SM agar with *Klebsiella aerogenes* and collected in sterile KK_2 buffer (16.5 mM, KH_2PO_4 , 3.8 mM K_2HPO_4 , 2 mM MgSO_4 , pH ~ 6.2) with 60 mM KCl added. They were pelleted at 200g for 3 min, washed three times in KK_2 and finally once in 5% Bonner's salts (100% is 0.6 g l^{-1} NaCl, 0.75 g l^{-1} KCl, 0.3 g l^{-1} CaCl_2). Cells at a density of $2.5 \times 10^5 \text{ cm}^{-2}$ were spread on 2 ml of 1.5% agar (Oxoid L28) containing 5% Bonner's salts, 250 $\mu\text{g ml}^{-1}$ streptomycin with or without 5 mM cyclic AMP, in a 5 cm Petri dish. Washed, sterile Cellophane (slit Visking dialysis tubing) held in perspex stretchers (each consisting of an inner ring fitting tightly into an outer one) was placed over the cells and air bubbles excluded. At the stated times of starvation the central area of agar and Cellophane was cut out and the cells fixed *in situ* with ice-cold fresh fixative (10 mM sodium-cacodylate, 0.5% OsO_4 , 0.5% glutaraldehyde, 1 mM MgCl_2 , 1 mM CaCl_2 , pH 6.8). After 20–30 min the cells were collected, washed three times with 10 mM sodium cacodylate, pH 6.8 and dehydrated in graded alcohols. The specimens were then infiltrated in acetone for 1 h, then in a 1:1 mixture of acetone and Spurr's resin for at least 6 h and finally polymerised in Spurr's resin at 60 °C for 24 h. Sections were stained with lead citrate and uranyl acetate and photographs taken of random fields of cells with a Siemens Elmiskop 1 electron microscope. Prespore vacuoles were seen in 28%, 37%, 26%, 2% and 4% of the cell profiles at 15, 20, 26, 37 and 52 h respectively of induction with cyclic AMP.

Fig. 2 Induction of prespore and stalk cells by cyclic AMP at low (a) or high (b) cell density. ○, Prespore cells, no cyclic AMP; □, stalk cells, no cyclic AMP; ●, prespore cells + cyclic AMP; ■, stalk cells + cyclic AMP. HMI cells were prepared and plated as described in Fig. 1. Those at high density ($2.5 \times 10^5 \text{ cm}^{-2}$) were overlaid with Cellophane but those at low ($5 \times 10^3 \text{ cm}^{-2}$) were not. At the stated times cells at high density were collected in KK_2 and disaggregated with Pronase/British anti-lewisite (BAL)¹³. Those at low density were detached by swirling in KK_2 lacking Mg^{2+} and then pelleted in siliconised centrifuge tubes for 10 min at 1,000g. In both cases the cells were then fixed in 60% methanol and fluorescently stained using absorbed anti-spore serum by a modification¹⁴ of the method of Hayashi and Takeuchi¹³. Cells were examined in a Zeiss Photomicroscope III equipped for epi-fluorescence.



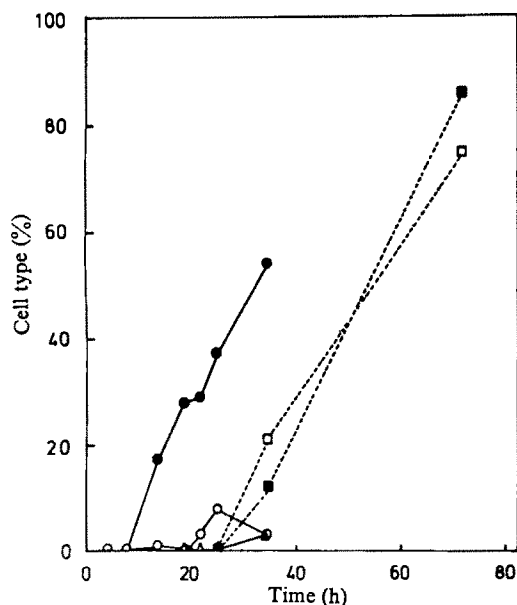


Fig. 3 Stalk and lack of prespore cell differentiation in cells at low density adjacent to high density 'helper' cells. ○, Prespore cells in low density cells over 'helper'; △, prespore cells in low density cells alone; □, stalk cells in low density cells over 'helper'; ●, prespore cells in 'helper'; ■, stalk cells in 'helper'. HMI cells were collected and plated on 5 mM cyclic AMP agar at a density of $2.5 \times 10^5 \text{ cm}^{-2}$ as described in Fig. 1. These 'helper' cells were overlaid with thin Cellophane (325 P, British Cellophane) held in a stretcher. More cells at low density (10^3 cm^{-2}) were then spread over the Cellophane. In control plates the 'helper' cells were omitted. At various times a cover slip was placed on the upper layer of cells and stalk cells scored above and below the Cellophane by microscopy. The low density cells were then detached from the Cellophane by swirling for 10 min with 50 mM Tris, 0.1% Pronase, 25 mM BAL pH 7.0¹³ and pelleted in siliconised centrifuge tubes at 1,000g for 10 min. 'Helper' cells were collected and both sets of cells stained as described in Fig. 2. The recovery of low density cells was normally 5–30% of those plated. Several controls indicate that there was not a selective loss of prespore cells during cell collection. First, similar results were obtained when cells were detached using the Pronase/BAL mixture diluted 1:10 or when they were scraped off in KK_2 and when they were pelleted at $\sim 10,000g$ for 30 s in a microfuge. Second, disaggregated high density cells, plated at low density could be recovered after 1 h with an efficiency of 14–50%. The losses occurring during cell collection did not in this case significantly change the proportion of prespore cells.

many must eventually transform into stalk cells. Support for this view comes from electron microscopic observations of cells taken at later times which show that many PSVs are in the process of breaking down, in a way resembling that described as occurring during the 'de-differentiation' of isolated prespore cells¹⁷. Such a transformation is also consistent with the observations of Town

and Stanford¹⁸ that prespore cells taken from slugs produce a high proportion of stalk cells (but no spores) in similar conditions.

As stalk cells are not inducible by cyclic AMP at low cell density we wondered if the same would be true for prespore cells. Figure 2a shows that this is indeed the case. From this result we conclude that a cell interaction must also be involved in the induction of prespore cells. We showed previously that stalk cell formation by cells at low density ('tester cells') was stimulated by the presence of a high density layer of cells ('helper cells') separated from them by a Cellophane membrane⁷. It was of interest therefore to see if the same was true for the formation of prespore cells. The results of a 'helper' experiment of this type, in which we assayed for stalk and prespore cells, is shown in Fig. 3. Stalk cells are induced in the helper and tester cells with near identical efficiency and kinetics. This is not true for prespore cells. Although a slight induction of prespore cells is consistently observed in the tester cells (compared to low density controls) it is much lower than in the helper cells themselves. Evidently the requirements for induction of prespore cells differs in some way from those for stalk cells.

In the light of these results it is apparent that the failure of wild-type amoebae to form spores when incubated with cyclic AMP under Cellophane at high cell density is not due to their failure to enter the prespore pathway but rather to some block in the maturation of prespore cells. Mutants can be isolated that do form spores in such conditions⁷. These mutants have presumably overcome the unknown block to spore maturation but might be expected to show the same requirement as wild-type amoebae for entry into the prespore pathway. We have, therefore, examined the ability of low density preparations of such strains to be helped to differentiate into stalk and spore cells by high density cells separated from them by a sheet of Cellophane. At the densities used the low density tester cells gave on their own 0–3% stalk cells and no spores. The results for five mutant strains presented in Table 1 show that as expected each is efficiently helped to make stalk cells but not to make spores.

From Table 1 it is evident that there is some stimulation of spore formation by the helper cells, especially when the density of the tester cells was $5 \times 10^3 \text{ cm}^{-2}$, the same density as used previously⁷. We have found (data not shown) that the efficiency of helping is not improved by using Cellophane treated with zinc chloride to increase its porosity. Further we have noted consistently that the spores formed as a result of helping are almost invariably very close to other cells, whereas most of the stalk cells are isolated (Table 1). Both these observations indicate that one of the requirements for entry into the prespore pathway involves a short-range cellular interaction, not required for stalk cell formation. The small stimulation of spore formation by helper cells is consistent with the idea that a low-molecular weight factor is required for both pathways of differentiation.

In conclusion, our results show that in appropriate conditions exogenous cyclic AMP can induce the formation of stalk cells and also the formation of prespore cells or even of mature spores.

Table 1 Enhancement of stalk and spore cell differentiation in low density mutant cells by the same cells at high density

Strain	% Differentiated cell type observed					
	Above cellophane $10^3 \text{ cells cm}^{-2}$			Below cellophane $2.5 \times 10^5 \text{ cells cm}^{-2}$		
	Total	Stalk	Isolated (as % of stalk)	Total	Stalk	Spore
HM9	15.5		90	0	11.9	26.7
HM9*	18.0		49	1.3	14.6	22.2
HM13	36.5		79	0.5	21.5	37.4
HM15	18.2		93	0	27.0	22.3
HM16	15.6		68	0	24.5	8.1
HM18	45.7		65	3.3	44.5	29

Cells of the mutants were collected as described in Fig. 1. High density cells were plated directly onto 5 mM cyclic AMP agar and overlaid with thin Cellophane as in Fig. 3. Cells of the same strain were spread on top at low density. After 3 d incubation at 22 °C a cover slip was placed on the top layer of cells and the cells scored above and below the Cellophane by microscopy. Strains HM13, 15, 16 and 18 were obtained by mutagenesis and selection from HM2 (a cobalt resistant strain temperature-sensitive for growth derived from HMI; R.R.K. & J. Trent, unpublished). Strain HM9 is clone 27 of the strain previously called *sci-1*.

*HM9 cells above Cellophane at a density of $5 \times 10^3 \text{ cm}^{-2}$.

Since cells under Cellophane become aggregation competent in the absence of cyclic AMP (R.R.K., unpublished observations) there must be a requirement for cyclic AMP in both pathways of differentiation beyond the stage of aggregation competence. We therefore regard with caution the idea that levels of cyclic AMP control the pattern of differentiation in *Dictyostelium*^{19,20}. We have, however, identified different requirements for the induction of stalk cells and of prespore cells (in the wild type) or spores (in the mutants). These differences could play a key part in controlling the pattern of differentiation in normal development.

We thank Dr David Forman for producing the antibody, James Vernon and Jennifer Trent for assistance and Dr Julian Gross for helpful discussions.

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Received 15 August; accepted 2 November 1977.

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Unintegrated ribosomal genes and their relation to position effect variegation in *Drosophila melanogaster*

THE variegated expression of genes is most often associated with a chromosomal rearrangement having one or both breakpoints within a heterochromatic region¹⁻³ and as a result, the genes lying near the breakpoint are usually suppressed. In *Drosophila melanogaster*, the genes coding for ribosomal RNA (rRNA) at the nucleolus organiser region are also situated in heterochromatin⁴. Ribosomal genes which are not integrated into the DNA of the chromosome have been found in *D. melanogaster* females heterozygous for an X chromosome inversion which transposes the nucleolus organiser to the tip of the X (refs 5, 6). It was proposed that these unintegrated ribosomal genes ($\sim 3 \times 10^8$ daltons) might be involved in position effect variegation⁵. This study was initiated to determine if a correlation exists between the presence of unintegrated ribosomal genes and the extent of position effect variegation in general. DNA from inversion-bearing male genotypes undergoing position-effect variegation was examined by sucrose gradient sedimentation and DNA-rRNA hybridisation. The resulting data further support a variegation association.

When the nucleolus organiser is moved by an inversion to the tip of the X chromosome, it has been shown to variegate⁷. Baker⁷ inferred from the lethality associated with *In(1)sc^L/0* and *In(1)sc^{S1}/0* males that these two X chromosome inversions were associated with a position effect suppression of ribosome synthesis. In the first series of experiments, the X chromosome

inversions used moved the nucleolus organiser to the tip of the X (*In(1)sc^{V2}*, *sc⁸*, *sc^{L8}*, and *sc^{S1}*). *In(1)sc^L*, on the other hand, has its proximal break distal to the nucleolus organiser and therefore left it at the base of the X. Oregon-R X chromosome was used as a wild-type control. DNA with a molecular weight of $\sim 5 \times 10^9$ was isolated from diploid brain and imaginal disks, and polytene salivary glands of third instar male larvae. Each male carried a specific X inversion and lacked a Y chromosome (*In(1)X/0*). As shown in Table 1, Oregon-R X/0 and *In(1)sc^L/0* males have no low molecular weight ribosomal genes in diploid tissue. rRNA hybridisation was within the high molecular weight DNA peak. Diploid larval tissue was analysed because of the lethality associated with some of the *In(1)X/0* genotypes⁷. The pattern of ribosomal gene integration found for adult flies has always been identical to that of the larval diploid tissue^{8,9}. All male genotypes which carry an inverted X with the right breakpoint between the nucleolus organiser and the centromere (*In(1)sc^{V2}*, *sc⁸*, *sc^{L8}*, and *sc^{S1}*) (Table 1) exhibit unintegrated ribosomal genes ($\sim 3 \times 10^8$ daltons) in increasing amounts. Of particular interest is the fact that *In(1)sc^{L8}/0* and *In(1)sc^{S1}/0* males produce the highest percentage of unintegrated ribosomal genes in diploid tissue and they also suffer the greatest lethality, since few males were observed to survive to third larval instar.

When the DNA from the polytene salivary glands was examined, all male genotypes had approximately 42% unintegrated ribosomal genes (Table 1). This percentage of low molecular weight genes in the salivary glands never varies, regardless of the genotype, and the finding has been discussed previously^{5,8}.

Mechanisms operating at the chromosomal level to affect gene expression may also be enhanced or suppressed by using suppressors of position-effect variegation³. One such locus is suppressor of variegation (*Su(var)*) in *D. melanogaster*. *Su(var)* is not a general modifier of position effect, it is limited to rearrangements involving specific heterochromatic regions (for example, *In(1)w^{m4}* and *rst³* compared with *In(1)sc^L*) (refs 10-13). In the second series of experiments, high molecular weight DNA was isolated from those inversion-bearing adult male genotypes which are affected by the *Su(var)* locus. The studies reported here were conducted using two alleles, *Su(var)* and *Su(var)⁺*, which can be maintained in homozygous stocks. *Su(var)⁺* enhances the variegation associated with *In(1)w^{m4}* and *In(1)rst³*, whereas *Su(var)* enhances variegation in *In(1)sc^L* (refs 3, 12, 14). When these suppressor alleles were associated with X chromosome inversions which enhance variegation, unintegrated ribosomal genes were present in all of the adult male genotypes analysed (Table 2). When variegation was suppressed by using *Su(var)* in association with *In(1)w^{m4}* and *In(1)rst³* and *Su(var)⁺* with *In(1)sc^L* (refs 3, 12, 14), no unintegrated ribosomal genes were found (Table 2).

Table 1 Percentage of unintegrated rDNA in male *D. melanogaster* of different genotypes

Genotype	Diploid brain and imaginal disks	Polytene salivary glands	No. of gradients analysed for each tissue
Ore-R X/0	0	42.1	3
<i>In(1)sc^L/0</i>	0	41.9	3
<i>In(1)sc^{V2}/0</i>	5.2	41.1	3
<i>In(1)sc⁸/0</i>	9.6	42.3	3
<i>In(1)sc^{L8}/0</i>	39.1	42.6	3
<i>In(1)sc^{S1}/0</i>	46.2	41.7	3

Drosophila melanogaster were labelled by growth at 23-25 °C on a low yeast medium as described previously^{8,9}. The medium contained 5 μ Ci ml⁻¹ ¹⁴C-methyl thymidine (50-60 mCi mmol, New England Nuclear). DNA isolation, sedimentation, and hybridisation of ³H-rRNA to the gradient fractions were as before^{8,9}. The *Drosophila* tissue culture rRNA used for the hybridisation had a specific activity of 309,000 c.p.m. μ g⁻¹. The stocks and inversions used in this study have been described previously^{5,8}. For genetic symbols and descriptions, refer to Lindsley and Grell¹⁰.

Table 2 Percentage of unintegrated rDNA in progeny adult males from female *D. melanogaster* of differing phenotype following inversion

Inversion	Mother	Progeny adult males	rDNA Unintegrated (%)
w^{m1}	Su(var) ⁺ /Su(var) ⁺	Su(var) ⁺ /Su(var) ⁺	17.8
w^{m1}	Su(var)/Su(var)	Su(var)/Su(var)	0
rst^2	Su(var) ⁺ /Su(var) ⁺	Su(var) ⁺ /Su(var) ⁺	16.2
rst^2	Su(var)/Su(var)	Su(var)/Su(var)	0
sc^1	Su(var)/Su(var)	Su(var)/Su(var)	18.2
sc^1	Su(var) ⁺ /Su(var) ⁺	Su(var) ⁺ /Su(var) ⁺	0

The *Drosophila* stocks and X chromosome inversions used have been described previously^{5,11-14}. The construction of genotypes was essentially the method of Spofford¹¹⁻¹⁴. Each inversion was placed opposite an attached X chromosome carrying the markers *y* (yellow) and *w* (white) and then introduced into companion stocks. The companion stocks were homozygous for *Su(var)* or for the *Su(var)*⁺ allele. This was done by a series of three crosses to replace the third chromosome of the inversion stock with *Ly Sb* (*Lyra*, *Stubble*) chromosome and then replace the latter with the *Su(var)* or *Su(var)*⁺ chromosome from coisogenic stocks. (The stocks carrying *Su(var)* or *Su(var)*⁺ were a gift of W. K. Baker, Chicago.) *Su(var)* and *Su(var)*⁺ females used for the crosses were tested for the *Su(var)* locus genotype by mating to *Dp(1:3)w^{m26.1-28.3}* males¹⁰⁻¹². For all the inversions studied, crosses between *Su(var)⁺/Su(var)⁺* or *Su(var)/Su(var)* were set up on ¹⁴C-methyl thymidine-labelled food. Adult sons carrying *Su(var)* or *Su(var)*⁺ and the inversion *In(1)w^{m1}*, *rst²*, or *sc¹* were picked from the progeny of each mating pair and their DNA was subjected to sucrose gradient sedimentation and hybridization to ³H-*Drosophila* rRNA. Three gradients were analysed for each inversion.

It seems that there is a diverse type of regulation of nucleolus organiser activity in *D. melanogaster*. This regulation can be expressed genetically as a variegated suppression of nucleolus organiser activity, or expressed physically in the form of unintegrated ribosomal genes. It is known that the physical structure of ribosomal DNA not only differs from the bulk of the DNA (ref. 15) but it also differs within the ribosomal DNA itself depending on the genotype^{5,6,8,9}. In *D. melanogaster* the nucleolus organiser, together with an undetermined extent of heterochromatin to either side of it, can also induce variegation in its neighbours when placed into euchromatin, for example near *et*, *l2*, or *in* (ref. 16). Inversions such as *In(1)sc^{V2}*, *sc^B*, *sc^{L8}* and *sc^{S1}* move a large amount of heterochromatin to the tip of the X chromosome, a condition which could favour variegation. Interestingly, Baker⁷ reported a decreasing viability index caused by suppression of the nucleolus organiser for *In(1)X/0* male genotypes (*sc^{L8}/0*:95; *sc^{V2}/0*:38; *sc^B/0*:14 or <1; and *sc^{S1}/0*:<1). In this study, I have found an increasing amount of unintegrated ribosomal genes as the apparent male viability decreased compared with female survival, and as the position effect increased. The viability of *In(1)sc^{L8}/0* and *In(1)sc^{S1}/0* males is partially restored by Y chromosomes with an altered nucleolus organiser and fully restored by unaltered Y chromosomes⁷. When these inversions were analysed in the presence of a Y, no unintegrated ribosomal genes were found^{5,6}.

In(1)sc^{S1}/0 male larvae were reported to have a 15% reduction in the amount of 18S and 28S rRNA (refs 17, 18). It was suggested that this reduction results from a suppression of transcription: perhaps it is the unintegrated ribosomal genes which cannot be transcribed efficiently. Furthermore, it has been suggested that the *Su(var)* locus might also affect a signal to begin message transcription¹⁴. It is not apparent, however, why the *Su(var)* locus should affect the nucleolus organiser region. The purpose of unintegrated ribosomal genes is still not understood. These genes have been postulated to be a result of chromosome pairing in inversion-bearing heterozygous females⁶. They have never been observed in adult males or in diploid tissue from male genotypes. The data presented here are the first to show that unintegrated ribosomal genes can occur in male genotypes of *D. melanogaster*. This report has also

presented two experimental situations in which position-effect variegation affects particular X chromosome inversions and also results in the presence of low molecular weight ribosomal genes. Little is known of the mechanism of position-effect variegation, but by studying chromosome rearrangements and factors which influence gene expression, a molecular explanation for the role of unintegrated genes in the variegation process may be found.

I thank Drs A. Gayler Harford and Thomas G. Sanders for reading the manuscript and for their helpful comments.

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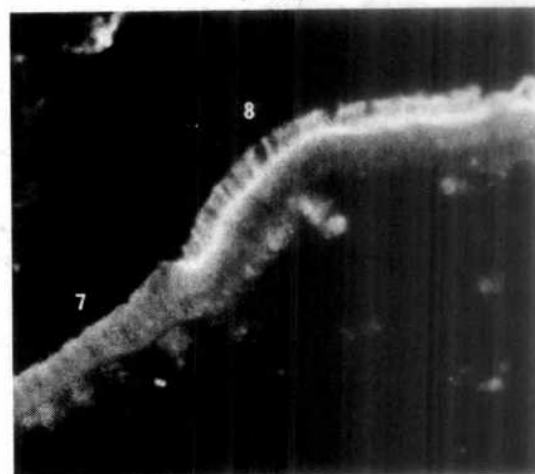
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Evidence for a 'mammalian' thyroglobulin in endostyle of the ascidian *Styela clava*

ATTENTION has focused on the protochordate endostyle since the demonstration that certain of its cells could bind iodine¹⁻⁴. That has been confirmed by ultrastructural studies⁴⁻⁸, while the presence of thyroid hormones and their precursors has been established in endostylar extracts⁸⁻¹⁰. These findings have been questioned because the blood¹¹ and tunic^{12,13} can bind large amounts of iodine. Moreover, both iodotyrosines and iodothyronines can be isolated from tunic extracts¹²⁻¹⁵. Barrington has suggested that iodine-binding by the tunic is an invertebrate process associated with the extracellular iodination of inert structural scleroproteins, whereas endostylar iodination involves intracellular glycoproteins⁸. Using indirect immunofluorescence¹⁶, with rabbit anti-thyroglobulin serum, I have now identified a

Fig. 1 Photomicrograph of zones 7 and 8 from the endostyle of *S. clava*. Note the brilliant apical fluorescence in the ciliated zone 8. The fluorescence at the tips of zone 7 cells rather less distinct. Zeiss epi-fluorescence, mercury vapour lamp, blue excitation ($\times 300$).



protein in the endostyle of the ascidian *Styela clava* that is immunologically identical to mammalian thyroglobulin.

Figure 1 shows the specific localisation of thyroglobulin in zones 7 and 8 of the endostyle of *S. clava*. The distinctly brighter fluorescence in zone 8 may reflect fundamental differences in the quantity and/or quality of the intracellular material. Equally it may simply be due to secretory material trapped at the bases of cilia. Routine controls using saline or non-immune serum proved negative.

Thus, in the regions of the endostyle that are known actively to bind iodine intracellularly, the protein to which it is evidently bound is immunologically indistinguishable from mammalian thyroglobulin. Unquestionably protochordates must be regarded as very specialised animals¹⁷, and the extent to which the iodine-binding cells themselves have also become specialised is difficult to ascertain.

Notwithstanding such difficulties, this latest evidence for a thyroglobulin-like glycoprotein in the iodine-binding cells, together with the earlier work cited here clearly makes the concept of the endostylar origin of vertebrate thyroidal cells more credible.

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Received 29 July; accepted 21 October 1977.

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Hormonal influences on ocular refraction in the rat

We report here that thyrotrophin (TSH) has a marked influence on ocular refraction in rats. In the clinical experience of one of us (C.C.K.-V.A.), adolescent myopia is not infrequently accompanied by increased height, suggesting a common, possibly hormonal, cause for both phenomena. No data on possible hormonal influences on eye growth or refraction could be found in the literature, but early anatomical studies showed that, during puberty, a growth spurt occurs in the eye after an early post-natal rapid weight increase¹⁻⁴. In a very detailed study, Weiss⁵ found relatively less growth of the sagittal eye axis as compared with the vertical and frontal diameters. Non-uniform growth rates along different eye axes might affect refraction.

In an attempt to assess hypophyseal influences on the eye, we studied the effects of some pituitary hormones on refraction and eye growth in rats. Seven-week-old ('adolescent') male Wistar rats were hypophysectomised and treated with a pituitary TSH preparation (Ambinon, Organon), which was dissolved in saline and injected subcutaneously twice daily, starting on the day after hypophysectomy. From preliminary experiments, a treatment period of 3 weeks was found to be optimal and was subsequently used unless indicated otherwise. Control rats were injected with saline alone. Ocular refraction was measured to the nearest dioptre by direct ophthalmoscopy after accommodation had been paralysed by a drop of 1%

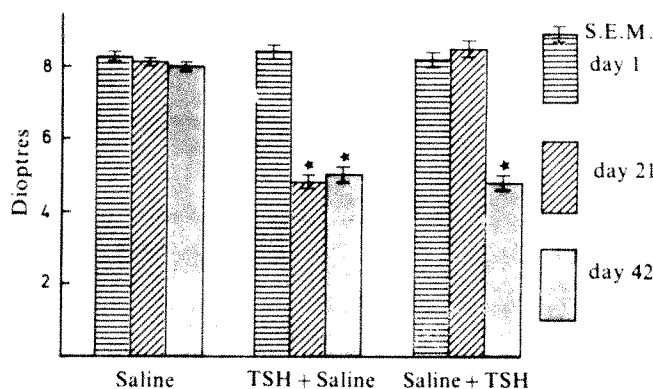


Fig. 1 Irreversibility of TSH effects on ocular refraction in hypophysectomised rats. Hypophysectomy was on day 1 and injections from day 2, twice daily 0.1 ml subcutaneously. Controls received saline from day 2 to day 42 ($n = 9$). TSH + saline: 2 IU TSH d^{-1} from day 2 to day 21, saline from day 22 to day 42 ($n = 9$). Saline + TSH: saline from day 2 to day 21, 2 IU TSH d^{-1} from day 22 to day 42 ($n = 8$). Autopsy was carried out on day 42. Averages of refraction in both eyes per rat were used for computation. Columns represent means \pm s.e.m. * $P < 0.01$, compared with intact controls, computed by analysis of variance.

atropine solution, 1 h before examination. The animal was restrained manually and the entrance of the superior temporal vein in the ocular fundus was brought into focus by interposing the appropriate ophthalmoscope lens. In Tables 1 and 2, 'refraction' refers to the strength of this compensating lens, expressed in dioptres. It should be stressed that the high positive values of the 'refraction' do not mean that rats have a physiological hypermetropia, as it is known that small-eyed animals tend to exhibit large hypermetropia^{6,8} attributable to a measurement artefact arising from the ophthalmoscopic reflection occurring in front of the receptors⁹. The eyes of most animals are, in fact, thought to be emmetropic. The term 'hypermetropia' is used here, however, for the sake of brevity. Ophthalmoscopy was always carried out by the same investigator, using an eye that could not accommodate. In the experiment of Table 1, the ophthalmoscopist did not know to which group an animal belonged. An unbiased adherence to such a 'blind' procedure was not always possible in other experiments, as operation scars, growth retardation and behavioural changes were unavoidable clues as to an animal's treatment. (In frequent 'blind' checks, however, the reproducibility of measurements was ascertained.) Refraction changes due to stress-induced accommodation, such as occurs in teleosts⁷, could be excluded since the rats were cycloplegic after atropinisation. One day after the last ophthalmoscopic examination, the animals were killed with chloroform. The eyes were enucleated and weighed on a torsion balance; the lenses were then extracted and also weighed.

The results in Table 1 show that TSH brings about a dose-dependent change in refraction. No significant changes in eye or lens weights were evident.

In order to assess whether endogenous TSH has comparable effects, its production was enhanced by thyroidectomy on 7-week-old rats in a subsequent experiment. As is shown in Table 2, 3 weeks later, refraction had changed markedly compared with intact controls, although eye and lens weights were unchanged. In contrast, control animals which had only been hypophysectomised, had markedly lower eye and lens weights, but their refraction remained normal. The results of this experiment were taken as evidence for a direct effect of TSH on the eye, also occurring in the absence of the thyroid gland.

We then investigated whether the ocular effect of TSH is reversible. One group of rats hypophysectomised at 7

Table 1 Effect of exogenous thyrotrophin on ocular refraction and eye weight in hypophysectomised rats

Treatment	n	Body weight (g)		Refraction (D)				Weight total 2 eyes (mg)	Weight total 2 lenses (mg)
		Day 1	Day 21	Day 1		Day 21			
				L	R	L	R		
Saline	11	201	190	8±0.6	8±0.8	8±0.6	8±0.5	216±6	62±3.8
TSH 1 IU d ⁻¹	11	200	202	8±0.8	8±0.8	5±0.7*	5±0.5*	221±7	62±4.7
TSH 2 IU d ⁻¹	11	199	210	8±0.3	8±0.0	4±0.8†	4±0.6†	222±6	64±4.7

In all experiments, rats were exactly 7-weeks-old on day 1, when hypophysectomy was performed. Injections of TSH or saline (0.1 ml subcutaneously twice daily) were made from day 2 until killing on day 21. D: Refraction (in dioptres) measured by ophthalmoscopy. Analysis of variance:

* Statistically significant, $P < 0.01$, from control values and from TSH 2 IU d⁻¹.

† $P < 0.01$, from control values and from TSH 1 IU d⁻¹. Means ± standard deviation.

weeks of age was treated with 2 IU TSH daily for 3 weeks and subsequently with saline for 3 weeks. In a second group, a crossover design was followed, whereby the animals received saline for 3 weeks, followed by 2 IU TSH d⁻¹ for 3 weeks. Hypophysectomised controls received saline for 6 weeks. At the end of the experiment, both groups treated with TSH showed the same change in refraction as seen before (Fig. 1). The animals treated first with TSH, then with saline, showed the expected refraction

were treated with either 2 IU TSH d⁻¹, or ACTH (1 U per 100 g body weight subcutaneously every alternate day), or a combination of the two hormones in the same dosages; controls received saline. Treatment lasted for 3 weeks and the results are shown in Fig. 2. TSH decreased hypermetropia; ACTH when given alone had no effect. When the two hormones were given simultaneously, however, the effect of TSH was not only blocked but even reversed, resulting in increased hypermetropia.

Table 2 Effect of exogenous thyrotrophin on ocular refraction and eye weight in hypophysectomised rats

Treatment	n	Body weight (g)		Refraction (D)				Weight total 2 eyes (mg)	Weight total 2 lenses (mg)
		Day 1	Day 21	Day 1		Day 21			
				L	R	L	R		
Intact	10	190	309	8±0.7	8±0.3	8±0.4	8±0.4	226±7	62 ±2.3
Hypophysectomised	13	189	173	8±0.9	8±1.0	8±0.4	8±0.5	212±7*	58 ±3.4*
Thyroidectomised	10	190	251	8±0.5	8±0.8	5±0.5*	5±0.5*	227±6	59 ±6.7

Either hypophysectomy or thyroidectomy was performed on days 1 and 2. Animals were left untreated until killing on day 21. Analysis of variance: * Statistically significant, $P < 0.01$, from control values.

alteration, indicating that these effects persist for at least 3 weeks after discontinuation of TSH treatment.

From Table 2 it follows that in intact animals, in spite of the presumably continuous presence of TSH, no decrease in hypermetropia occurs between 7 and 10 weeks of age. Obviously, in such rats, compensatory influences are operative. In a first analysis of such a compensation by other pituitary hormones, 7-week-old hypophysectomised rats

In rats, the Harderian gland is the major retrobulbar constituent of orbital contents, having about the same weight as the eye⁸. Changes in gland size might cause deformation of the eye bulb and hence refraction changes. Therefore we looked for parallelism between changes in Harderian gland size and refraction under the influence of exogenous or endogenous TSH. Three weeks after hypophysectomy there was a marked atrophy of the Harderian gland, but refraction remained normal (Fig. 3). After TSH treatment of hypophysectomised animals, the decrease in hypermetropia was not accompanied by a change in gland atrophy. The rise of endogenous TSH after thyroidectomy, however, does not affect Harderian gland size, it only changes refraction. We therefore conclude that there is no causal relationship between Harderian gland size and refraction.

In conclusion, TSH induces changes in refraction of the rat eye by a process involving neither thyroid hormone nor the Harderian gland. Experiments to elucidate the physical, morphological and biochemical mechanisms causing refraction changes are in progress, with special attention being paid to corneal radius and refraction, and lenticular refraction and biochemistry.

No literature data have come to our knowledge on modifications of ocular refraction in the rat. As to eye growth in the rat, it is known that after very rapid growth in early life, the weight increases at a slower rate during the remaining lifespan⁹⁻¹¹. Although some factors such as food, care and physical condition have been shown to influence relative and absolute eye weight⁹, no specific hormonal factors have until now been held responsible for such phenomena. If TSH can be shown to affect refraction in man in a similar way to that in the rat, the data presented here open up

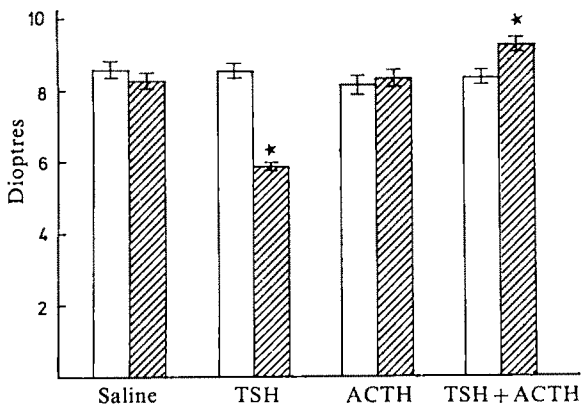


Fig. 2 Blockade by ACTH of TSH effect on ocular refraction in hypophysectomised rats. Hypophysectomy was on day 1. TSH (2 IU d⁻¹) and saline were injected twice daily. ACTH (1 IU per 100 g body weight) was injected every other day. No significant differences in eye weights were found. * $P < 0.01$, compared with saline-treated controls, computed by analysis of variance. Six animals in each group. Open columns, day 1; hatched columns, day 21.

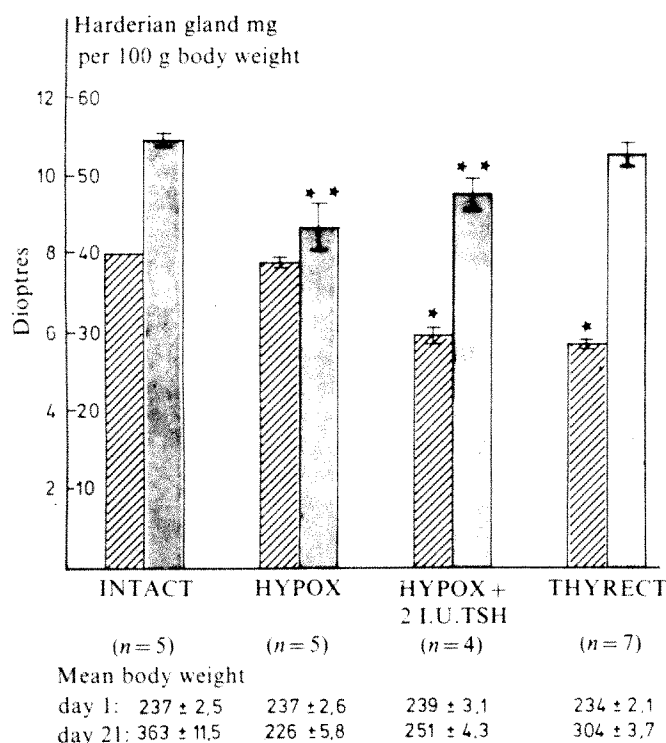


Fig. 3 Effect of exogenous and endogenous TSH on Harderian glands and refraction in rats. Hypophysectomy (hypox.) or thyroidectomy (thyrect.) was performed on day 1. TSH treatment (2 IU d⁻¹) was given from day 2 until killing on day 21. Left column of each pair: refraction data as determined shortly before autopsy; right column: mg Harderian gland per 100 g body weight. **P* < 0.05; ***P* < 0.01.

interesting perspectives for pharmacological treatment of juvenile refraction aberrations.

We thank D. Laman for technical assistance. This investigation was supported by a grant from the Netherlands Ophthalmic Research Institute. Ambinon was supplied to us by Organon International BV, The Netherlands.

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Received 22 March; accepted 25 October 1977.

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Three forms of delayed skin-test response evoked by mycobacteria

Koch¹ first reported that when guinea-pigs which had been infected 4–6 weeks previously with tuberculosis were challenged intracutaneously (i.c.) with a small number of virulent organisms, there was induration and necrosis within 48 h, and localisation of the challenge dose. This phenomenon (classical tuberculin-type hypersensitivity) has often been regarded as a direct correlate of protective

immunity. But, the same challenge dose was not localised if injected intramuscularly (i.m.). This raised the possibility that 'protection' from the i.c. challenge was a trivial consequence of necrosis and sloughing of the infected skin. The controversy was highlighted by Wilson² who noted that pre-immunised guinea pigs showing an exquisite degree of hypersensitivity at the time of a small i.m. challenge with virulent *Mycobacterium tuberculosis* died more than 40 d before unimmunised controls, whereas weakly hypersensitive animals showed protection. We describe here a form of delayed foot-pad response which is evoked by all the non-pathogenic mycobacteria studied, but differs from the Koch-type response in timing and suppressor cell activity. It is not seen following injection of pathogens, and it is suggested that failure to evoke this response may account for their pathogenicity.

The existence of delayed hypersensitivity responses to mycobacteria, differing from the day 28 'Koch-type' was revealed by skin testing at intervals after injecting 10⁸ mycobacteria (see Fig. 1 legend) into groups of 20 or 40 BALB/c mice of either sex. Foot-pads were measured before and 24 h after challenge with 5 µg (protein) in 20 µl of saline of a 0.22-µm membrane filtered ultrasonic³ of the same organisms. Delayed hypersensitivity was calculated as per cent swelling in groups of 5–10 mice. All of eight species (provided by Dr J. L. Stanford) known to be non-pathogenic for the mouse (unpublished observation) gave curves of the type shown for *M. nonchromogenicum* in Fig. 1, with a pronounced peak at day 10. The mouse pathogens *M. ulcerans*, *M. kansasii*, and some *M. avium* strains gave a smaller peak between days 5 and 7, but the day 10–11 peak was absent. Similarly, relatively mouse virulent⁴ BCG strains such as Pasteur gave this pathogen-type curve, whereas Glaxo BCG which is of low mouse virulence⁴ gave a powerful day 10 peak. All the organisms gave rise to a later response which resembled the classical Koch type in that the peak occurred between 4 and 6 weeks.

None of the three peaks occurred in T lymphocyte-deprived mice injected with 10⁸ *M. nonchromogenicum* or BCG Pasteur. The day 10 response was readily transferred to normal recipients by the intravenous injection 2 × 10⁷ cells from nodes draining the injection site. This transfer was completely abrogated by killing the donor T cells with anti-Thy 1.2, and complement.

Treatment of the mice with intraperitoneal cyclophosphamide, 200 mg per kg body weight 24 h before injection of the bacilli increased the day 6–7 response, without affecting the day 10 peak, if present, or the subsequent day 17 trough. This observation suggested that the day 6–7 peak represented the 'Jones-Mote' phenomenon which typically occurs at 7 d, and is known to be induced by most, if not all mycobacterial species⁵. A regulator of this phenomenon has been reported to be a cyclophosphamide sensitive lymphocyte, in both the guinea pig⁶ and the mouse⁷.

The nature of the 'trough' occurring 17 d after subcutaneous (s.c.) injections of non-pathogenic species (Fig. 1) was investigated by transferring 0.5 ml of fresh serum, or 1.5 × 10⁷ cells from nodes draining the injection site of such day 17 animals, into recipients at other points on the time course (Table 1). Thus normal animals, or mice which had received organisms s.c. 6, 10 or 28 d earlier, received day 17 cells, or serum, or both 1 h before being foot-pad tested.

Neither day 17 cells, nor day 17 serum had demonstrable effects on the foot pad-test responses of control, or day 6 (Jones-Mote) mice. But, day 17 cells suppressed both the day 10, and the day 28 peaks, particularly the latter. Recovery from this suppression was partial by 7 d but complete 14 d later (Table 1).

Day 17 serum enhanced the day 10 peak, but was without

Table 1 Delayed skin-test response to *M. nonchromogenicum*

Treatment of recipients	Controls	Foot-pad responses in recipients (% swelling at 24 h)			Day 28 retested 14 d later
		Day 6	Day 10	Day 28	
None	5.7±4.4	20.3±4.9	38.6 ± 6.2	35.9 ± 12.0	33.1 ± 13.0
1.5 × 10 ⁷ day 17 cells*	4.3±1.6	20.5±4.4	28.3† ± 4.1	9.1† ± 5.4	30.4 ± 7.5
0.5 ml day 17 serum*	5.6±4.4	28.3±7.2	66.9‡ ± 13.9	35.5 ± 11.2	ND
1.5 × 10 ⁷ cells and 0.5 ml serum*	ND	ND	ND	23.0 ± 8.4	ND

Effect of cells or serum taken from BALB/c mice 17 d after 10⁸ *M. nonchromogenicum* s.c., on the foot-pad responses to 5 µg of antigen, of normal mice, or mice which had received 10⁸ organisms s.c., 6, 10 or 28 d earlier. (10 mice in each group.) ND, not determined.

*Injected intravenously 1 h before the foot-pad test.

†Significantly different from control group. ($P < 0.001$, t -test).

‡Significantly different from control group. ($P < 0.025$).

effect on the day 28 response. But, in subsequent experiments, when transferred into day 28 animals which also received day 17 cells, the serum partially reversed the suppressive effect of the cells, implying that when the serum factor enhanced it did so by opposing suppressor cells, rather than by a direct influence on effector cells. It will be important to find whether this serum factor is similar to that which accompanies tissue-damaging reversal reactions in human leprosy⁸.

Neither day 17 cells or serum had any significant effect on the delayed hypersensitivity responses to sheep red blood cells, or picryl chloride in appropriately immunised mice.

The major observation here, is that only pathogenic mycobacteria either do not evoke, or are able to suppress the day 10 type of T lymphocyte-dependent response. This is circumstantial evidence for a protective role for this response, which, in timing resembles delayed hypersensitivity evoked by other intracellular parasites such as *Listeria monocytogenes*⁹, and *Leishmania*¹⁰. Thus the mechanism of immunity to mycobacteria in the subclinically infected individual may not be the 4–6 week Koch type of hypersensitivity as has often been assumed^{2,11} but may be very similar to that involved in protection from other intracellular organisms. This day 10 peak is accompanied by, and eventually switched off by suppressor

or 'regulator' cells (day 17) the effects of which are modulated by a serum factor. The later day 28 response differs not only in timing, but also in that this type of suppressor cell seems to be absent. This 4–6 week 'Koch-type' of hypersensitivity might be due to a failure of the normal homeostatic suppressor cell mediated regulatory mechanisms. (An ability of mycobacteria to interfere with suppressor cell production would explain the efficiency with which mycobacterial adjuvants will induce auto-immune responses.)

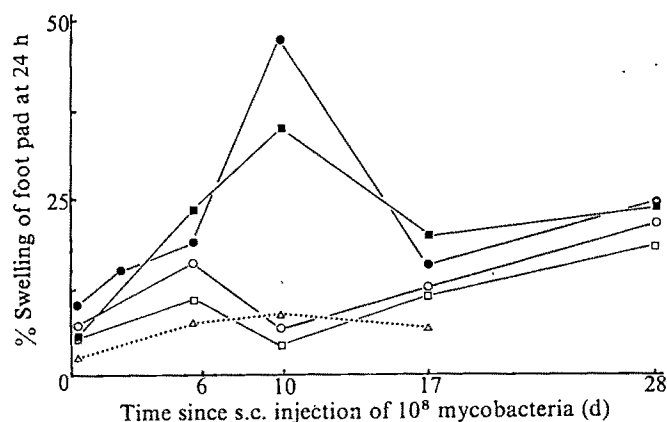
The 4–6 week delay before the appearance of the 'Koch-type' hypersensitivity is explained by the observation that its development is a two stage process. The first stage, which is not antigen specific, requires 2–3 weeks of exposure of lymphoid tissue to mycobacterial components. Thus when sheep red cells (SRBC) were injected into sites previously primed with Pasteur BCG which gives a pathogen-type of curve and fails to evoke day 10 peak, maximal potentiation of the response was seen if the SRBC were injected 2 weeks after the BCG¹². No potentiation was seen if both injections were given at the same time. Similarly, dead mycobacteria, or killed organisms, which traditionally do not induce 'Koch-type' hypersensitivity, give powerful responses if injected twice into the same site with a 2-week interval (data not shown).

Live, virulent organisms do not need to be injected twice, because having escaped the day 10 response by suppressing or failing to evoke it, they continue to proliferate and release antigen which reaches the lymphoid tissue after the first stage of non-antigen specific 'conditioning'. The resulting unregulated, day 28 or 'Koch-type' tissue-destructive hypersensitivity was probably responsible for the accelerated death of Wilsons guinea pigs, and this would help to explain cavitating pulmonary tuberculosis and reversal reactions and nerve damage in tuberculoïd leprosy.

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Fig. 1 Mycobacteria were collected from early log phase cultures on solid Sauton's medium, except for Glaxo BCG which was collected by centrifugation from Glaxo liquid production medium. Organisms were dispersed using a 1-ml syringe, washed three times in saline, centrifuged at 600g for 2 min to remove aggregates of organisms, and 5 µl of suitable dilutions spread over a known area of a microscope slide were counted using an auramine stain and a fluorescent microscope. Mice received 10⁸ organisms s.c. in the base of the tail. Delayed hypersensitivity was assessed by the foot-pad test as described in the text. There are 5–10 mice per point. *M. nonchromogenicum* (●), Glaxo BCG (■), Pasteur BCG (□), *M. kansasii* (○), in normal mice, and *M. nonchromogenicum* in adult thymectomised, 750-rad irradiated, bone-marrow reconstituted mice (△).



Received 13 July; accepted 31 October 1977.

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Influenza A viruses of the H2N2 subtype are lymphocyte mitogens

STUDIES of the effects of viral infections have largely been concerned with intracellular interactions between viral and cellular functions. There is increasing realisation that the cell surface has a major role in cellular function and various cell surface active substances, for example, concanavalin A (con A)¹ and phytohaemagglutinin (PHA)², exert profound effects on cellular metabolism. As the cell surface possesses receptors specific for various viruses and viral antigens, it might be expected that certain viruses could affect cellular function as a result of cell surface interaction. Proliferation is a characteristic response of lymphocytes exposed to these cell surface active compounds. This response can be readily quantitated using ³H-thymidine incorporation. We report here that influenza viruses of the H2N2 subtype are potent mitogens for both B and T lymphocytes³. This finding arose during studies aimed at developing assays for cell-mediated immunity to influenza virus infection⁴⁻⁷. We observed that spleen cells from normal mice showed increased levels of ³H-thymidine incorporation when exposed *in vitro* to the A/Singapore/1/57 virus. This model system should provide insight to questions of lymphocyte triggering and bring attention to this aspect of cell-virus interaction.

As illustrated in Fig. 1, maximum stimulation of normal mouse splenic lymphocytes occurred 48 h after the addition of 10 μ l of A/Singapore virus from a stock containing 10^{7.3} 50% egg infectious dose (EID₅₀) per 0.1 ml. Experiments were designed to determine whether the mitogenic activity of the egg grown stock was virus-associated and, if so, whether it was dependent on virus infectivity. A/Singapore virus was therefore sedimented in an ultracentrifuge and the supernatant and viral pellet were separated. Mitogenic activity was associated with the viral pellet (stimulation ratio (SR) of 3.9) but not with the supernatant fluid (SR of 0.9). In addition, the A/Singapore virus stock was shown to be free from bacterial contamination and had no detectable endotoxin (lipopolysaccharide), a known B cell mitogen, when assayed by the limulus lysate test (sensitivity < 0.5 ng ml⁻¹). Experiments were performed with live (10^{7.3} EID₅₀ per 0.1 ml) and ultraviolet-inactivated (< 10^{6.5} EID₅₀ per 0.1 ml) virus. These experiments indicated that virus infectivity was not required for stimulation, the SR of live and inactivated virus being 11.6 and 18.3 respectively.

The nature of the cells responding to A/Singapore virus was then evaluated. The response of untreated spleen cells was compared with that of either spleen cells depleted of T cells by treatment with anti- θ serum plus complement or

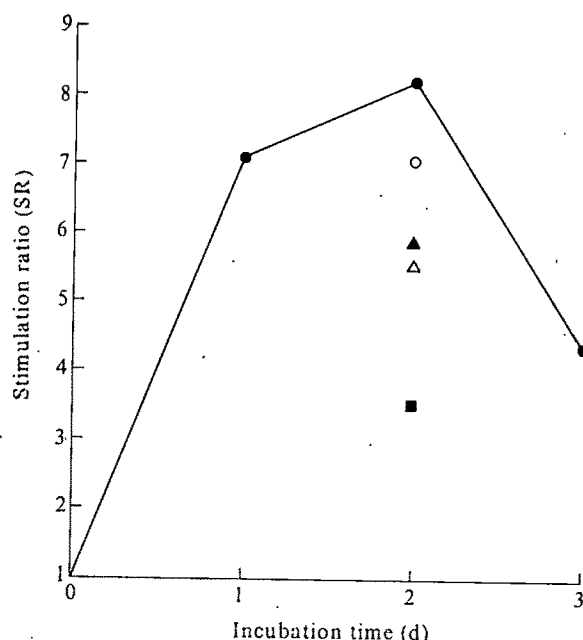


Fig. 1 Effect of time of incubation and dose of A/Singapore virus on *in vitro* proliferation of normal BALB/c splenic lymphocytes. ▲, 0.1 μ l; ●, 10 μ l; △, 30 μ l; and ■, 60 μ l of the A/Singapore virus; ○, 5 μ g con A. In these experiments normal spleens were removed from various animals and single cell suspensions were prepared. Erythrocytes were lysed by exposure of the spleen cells to buffered ammonium chloride solution⁸. Lymphocytes were resuspended to a concentration of 3×10^6 viable cells per ml in RPMI 1640 containing 4% heat-inactivated foetal bovine serum and 2×10^{-4} M 2-mercaptoethanol. Volumes of 0.2 ml of lymphocyte suspension were added to wells of microtitre plates (Microtest II, Falcon). A/Singapore virus was added in varying volumes to quadruplicate samples of normal lymphocytes. These studies were performed using an egg-passaged pool of A/Singapore virus with an egg-infectivity titre of 10^{7.3} EID₅₀ per 0.1 ml. The microtitre plates were incubated for 44 h at 37 °C in a 5% CO₂-95% air atmosphere. At this time 1 μ Ci of ³H-thymidine (specific activity, 6 Ci mmol⁻¹) (Schwarz/Mann) was added per well. After 4 h of further incubation, the cells were collected on to glass fibre filter strips using a MASH II harvester (Microbiological Associates) and the incorporation of ³H-thymidine determined by scintillation counting. Mean incorporation was determined for each group and a stimulation ratio (SR) derived by dividing the mean c.p.m. incorporated in the presence of virus by the mean c.p.m. incorporated in the absence of virus.

spleen cells depleted of B cells by passage through nylon fibre columns according to the method of Julius *et al.*¹⁰. As indicated in Table 1, both T- and B-enriched lymphocyte populations were stimulated by the A/Singapore virus. The responsiveness of B lymphocytes was confirmed in studies using splenic lymphocytes obtained from T-cell deficient nude (athymic) mice (Table 2).

Blastogenic responses were observed with lymphocytes from several mouse strains tested, including those from BALB/c mice raised in a germ-free environment. In addition, lymphocytes from human peripheral blood and spleen cells from Hartley guinea pigs and New Zealand white rabbits were stimulated by A/Singapore virus (Table 2).

Additional influenza viruses were tested for mitogenic activity. Splenic lymphocytes from normal BALB/c mice showed significantly increased levels of ³H-thymidine incorporation when exposed *in vitro* to all influenza viruses of the H2N2 subtype tested (Table 3). Influenza viruses of other subtypes produced little or no stimulation.

These studies indicate that several egg grown influenza viruses of the H2N2 subtype possess significant mitogenic activity for normal T and B lymphocytes. In addition, A/Japan/305 virus grown in a canine kidney cell line (MDCK) possessed mitogenic activity similar to that of the egg-grown virus. The observed blastogenesis is unlikely to be due to a specific immune response associated with cross-

Table 1 Mitogenic effect of A/Singapore influenza virus on T and B enriched lymphocyte populations

Treatment of normal BALB/c spleen cells	Mean stimulation ratio in cultures containing A/Singapore virus	
	A/Singapore virus	Con A
Untreated	6.2	2.7
Passed through nylon fibre column	4.4	16.7
Treated with complement	2.4	4.1
Treated with anti- θ plus complement	2.4	1.1

Quadruplicate cultures of treated and untreated spleen cells from BALB/c mice were incubated either in the presence or absence of A/Singapore virus as described in Fig. 1. A stimulation ratio for each of the four replicate cultures containing virus was calculated by dividing the counts of ³H-thymidine incorporated in each culture by the mean of the counts of ³H-thymidine incorporated in the quadruplicate cultures not containing virus (approximately 10⁴ c.p.m.). The value shown is the mean of the four calculated stimulation ratios. In all experimental groups, the range of the stimulation ratios observed in quadruplicate samples were within 10% of the mean value shown. Similar results were observed in repeat experiments.

Table 2 Mitogenic effect of A/Singapore influenza virus on normal lymphocytes

Source of lymphocytes tested for mitogenic activity	Mean stimulation ratio in the presence of A/Singapore virus
Nude mouse spleen	3.0
C3 H mouse spleen	10.2
Germfree mouse spleen	13.2
Human peripheral blood*	10.6
Guinea pig spleen	4.5
Rabbit spleen	3.2

The stimulation ratios shown are those calculated from four replicate lymphocyte cultures containing A/Singapore virus as described in Table 1. The mean counts of ^3H -thymidine incorporated in quadruplicate cultures not containing virus had a range between 10^3 and 10^4 c.p.m. In all experimental groups, the range of the stimulation ratios observed in quadruplicate samples were within 10% of the mean value shown. Similar results were observed in repeat experiments.

*Healthy adults aged 30–35 yr.

reacting microbial antigens because of the responsiveness of lymphocytes from germ-free mice. Nor is it likely to be due to virus infection of lymphocytes as ultraviolet-inactivated virus retained mitogenicity. Rather the data favour a cellular effect due to virus binding to the lymphoid cell surface. The H2N2 subtype has several distinguishing characteristics which separate those viruses from other influenza subtypes. The most notable feature and basis for classification of the subtype is the possession of the H2 haemagglutinin glycoprotein on the viral envelope. As all A type influenza viruses possess similar matrix and nucleoproteins and because viruses possessing the same neuraminidase (N2) but a different haemagglutinin (H3) did not stimulate lymphocytes (Table 3), these results suggest that the H2 glycoprotein is responsible for this mitogenic activity. Analyses of differences between H2N2 and other influenza viruses should give insight into the molecular basis of the cell surface interaction leading to lymphocyte transformation.

This report presents data that clearly demonstrate that H2N2 influenza viruses are mitogenic for lymphocytes. The possible effects of H2N2 influenza viruses on other types of cells remains to be determined. Another important question is whether other viruses exist with a mitogenic capacity

Table 3 Mitogenic effect of H2N2 influenza viruses on normal lymphocytes

Viruses tested for mitogenic activity		Mean stimulation ratio
A/Singapore/1/57	(H2N2)*	7.7
A/Japan/305/57	(H2N2)	8.9
A/Japan/170/62	(H2N2)	11.5
A/Taiwan/1/64	(H2N2)	10.7
A/Ann Arbor/7/67	(H2N2)	8.9
A/Tokyo/3/67	(H2N2)	6.4
A/Cornell/1001/67	(H2N2)	9.4
A/New Jersey/8/76	(Hsw1N1)	1.2
A/PR/8/34	(H0N1)	1.0
A/FM/1/47	(H1N1)	1.0
A/Port Chalmers/1/73	(H3N2)	1.1
A/England/42/72	(H3N2)	1.2
A/Victoria/3/75	(H3N2)	0.6

Quadruplicate cultures of spleen cells from BALB/c mice were incubated in the presence of several influenza A virus subtypes as described in Fig. 1. A mean stimulation ratio for each of the four replicate cultures containing virus was calculated as described in Table 1. The mean counts of ^3H -thymidine incorporated in quadruplicate cultures not containing virus was 2×10^4 c.p.m. In all experimental groups, the range of the stimulation ratios observed in quadruplicate samples were within 10% of the mean value shown. Similar results were observed in repeat experiments.

*Major surface antigens: haemagglutinin (H), neuraminidase (N).

similar to H2N2 influenza viruses. Finally the biological relevance of virus-stimulated proliferative lymphocyte responses needs to be clarified. Compared to resting lymphocytes, mitogen-activated lymphocytes are more permissive for growth of a wide variety of RNA and DNA viruses^{11–13}. In addition mitogen-induced proliferation has been shown to cause the activation of endogenous xenotropic type-C RNA virus from murine spleen cells^{14–15}. The possible contribution to disease pathogenesis due to mitogenic activity of viruses clearly deserves more study.

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Received 8 September; accepted 1 November 1977.

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Antibody to nuclear ribonucleoprotein penetrates live human mononuclear cells through Fc receptors

It is commonly accepted that antibodies do not penetrate living cells. In only one study anti-purine and anti-nucleoside antibodies were found to penetrate fertilised sea urchin eggs and modify their development¹. Such penetration has been considered unusual and the addition of anti-DNA antibodies does not affect mammalian tissue cells in culture². Direct immunofluorescence of skin biopsies of patients with mixed connective tissue disease (MCTD) using fluorescent anti-IgG has occasionally shown speckled intranuclear fluorescence^{3–5} but it is doubted that IgG entered the cells while still viable. Patients with MCTD have high titres of antibody to nuclear ribonucleoprotein (RNP)^{6,7} which also gives a nuclear speckled pattern on cell substrates in direct immunofluorescence⁸. Should the antibodies to cellular components and nucleic acids which occur in autoimmune diseases be able to penetrate living cells, a novel mechanism of immunologically mediated damage and/or dysfunction could operate. We show here that anti-RNP IgG can penetrate viable human mononuclear cells (MNC), by their surface Fc receptor, and react with their nuclear RNP.

To study the possible penetration of antibodies into viable cells we used an anti-RNP antibody present at high titres (1:16,384,000), as detected by a haemagglutination assay⁷, in the serum of a patient who fulfilled our criteria for MCTD⁹. This serum was unreactive with ribonuclease-treated extractable nuclear antigen (ENA), gave a single precipitin line on Ouchterlony analysis against untreated ENA, indicating its sole reactivity with RNP, and gave the characteristic speckled pattern on indirect immunofluorescence using frozen section of rat stomach as a cell substrate (Fig. 1a). It contained $106,680 \text{ mg l}^{-1}$ of IgG, 2,680 of

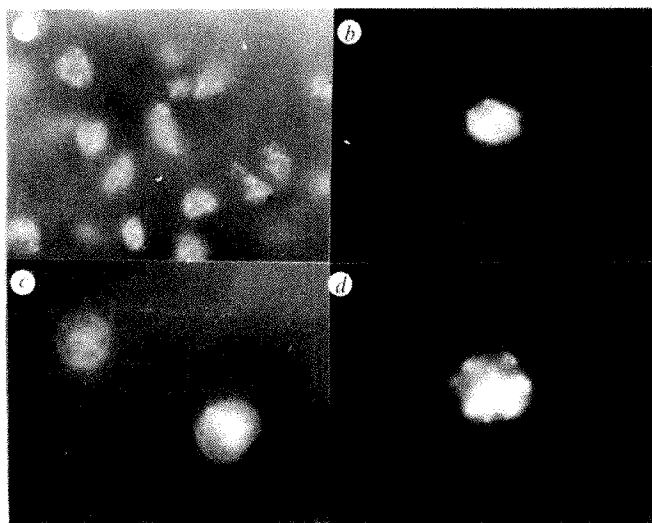


Fig. 1a. Nuclear speckled pattern under epifluorescent microscopy of a frozen section of rat stomach stained with the fluorescein-labelled anti-RNP IgG antibody. The sections were incubated for 25 min with the antiserum, washed for 20 min with isotonic saline under constant agitation, mounted with 0.1 M Glycine buffer, pH 8.6 with 2 parts glycerol, and read. *b*, Speckled nuclear pattern of a viable MNC after incubation with fluorescein-labelled anti-RNP IgG antibody. Viability was controlled by Trypan blue exclusion. *c*, Two viable MNC under fluorescent microscope after incubation with the fluorescent anti-RNP antibody. The lower one shows intranuclear fluorescence as well as three dots of cytoplasmic fluorescence. The upper one shows membrane fluorescence. *d*, A viable MNC, probably a macrophage, shows lumps of intracytoplasmic fluorescence after having been incubated with the fluorescent anti-RNP antibody. All photographs were taken with a C35 47 6070 Zeiss microscope camera using tri-X-pan Kodak film at original magnification of 800 \times .

IgA, and 2,520 of IgM as determined by radial immunodiffusion in commercial antibody-agar plates (Behringwerke). The IgG anti-RNP antibody-containing fractions obtained in Sephadex G-200 column fractionation had a titre of anti-RNP antibody of 1:1,048,576 at a protein concentration of 42 mg ml⁻¹. This IgG was tagged to fluorescent isothiocyanate by conventional methods and was absorbed with rat liver powder to eliminate unspecific fluorescence. To avoid the lymphocytotoxic activity found in MCTD sera¹⁰ we also absorbed it repeatedly with human brain cortex, which is known to share antigenic determinants with lymphocytes¹². The brain cortex was previously treated with ribonuclease-A (Sigma, lot 115C-0008) in order to avoid absorption of the anti-RNP antibody by RNP present in this tissue. Following this treatment, the mean lymphocytotoxic activity in a panel from 10 normal human donors in a modification of the microcytotoxicity assay of Terasaki and McClelland^{10,11} was 15%, which is close to that of normal sera¹¹, while the anti-RNP antibody titre remained unchanged. Viability of human peripheral blood mononuclear cells (MNC), obtained from normal donors by the Ficoll-Hypaque method of Böyum¹¹, was tested by Trypan blue exclusion after incubation with the antibody and was found to be 84.6%, which was acceptable for our studies. All subsequent studies were done using this absorbed, fluorescent anti-RNP IgG, and were controlled for viability by Trypan blue exclusion.

One fifth of all viable MNC from five normal subjects showed intranuclear fluorescence (Table 1) with the characteristic speckled pattern of anti-RNP when incubated 60 min at 37°C with the fluorescent antibody (Fig. 1b). Longer incubation did not increase the number of viable cells with intranuclear speckled fluorescence. Membrane fluorescence (Fig. 1c) was found in 17% of viable cells but seldom concurred with intranuclear fluorescence (Table 1). Capping was seen occasionally. Intracytoplasmic fluorescence seen in 6% of cells (Fig. 1d), was seldom associated with intranuclear fluorescence.

To determine if membrane and cytoplasmic fluorescence could represent steps in the pathway to the nucleus of the anti-RNP antibody, sequential studies were done after increasing in-

cubation times (Table 1). Within 1 h there was a stepwise increase of cells bearing intranuclear fluorescence as well as of those with membrane fluorescence. Inasmuch as fluorescence in these two locations increased in a parallel fashion, it could not be interpreted as steps of the same process. The different proportions of cells bearing nuclear fluorescence, as compared to those bearing membrane fluorescence, when enriched subpopulations were studied (*vide infra*), also suggest that these two different locations of fluorescence occur in different cell subpopulations rather than being stages of antibody penetration. Conversely, cytoplasmic fluorescence decreased along with intranuclear fluorescence in experiments in which penetration of the antibody was blocked (Table 1), suggesting that cytoplasmic fluorescence is due to the antibody on its way to the nucleus.

Five per cent of those viable MNC which adhered to glass wool columns or Petri dishes and stained with myeloperoxidase, indicating them to be predominantly macrophages¹³, were found to have intranuclear staining after incubation with the antibody, while 14% of non-adherent viable MNC showed such intranuclear staining. It therefore seems that the MNC into which the antibody penetrates are primarily lymphocytes, although it may also enter macrophages. To find into which lymphocyte subpopulation the antibody penetrates we prepared cell suspensions enriched for either B or T lymphocytes by a double Ficoll-Hypaque gradient method¹⁴. Suspensions containing predominantly B lymphocytes showed considerably greater percentages of intranuclear staining than those containing predominantly T lymphocytes (Table 1).

The percentages of viable MNC showing intranuclear staining by the labelled anti-RNP antibody are similar to those of MNC bearing receptors for the Fc fragment of IgG¹⁵. To determine if the Fc receptor-bearing MNC are those into which the antibody enters, they were incubated with antibody-coated chicken erythrocytes¹⁶ and the fluorescent anti-RNP antibody simultaneously. Fluorescent staining of nuclei was practically limited to cells forming Fc rosettes.

As the question of viability is crucial in establishing the significance of these findings, and because both Trypan blue exclusion and rosette formation may not be reliable indicators of cell viability at a given moment, we cultured in a 5% CO₂ atmosphere normal MNC pre-incubated with the labelled antibody and shown to have it internalised. Presence of cells bearing speckled intranuclear fluorescence after 24 h of culture confirmed their viability when penetration of the antibody occurred.

Since the antibody penetrates primarily into Fc receptor-bearing cells, this receptor could have a role in the uptake and entrance of the antibody. Normal human MNC were incubated for 1 h with heat-aggregated normal human Cohn's fraction II¹⁷ at concentrations ranging from 16 to 260 μ g per 10⁶ cells before incubation with the labelled antibody (Table 1). Abrogation of antibody penetration by pre-incubation with aggregated γ -globulin was dose related (Table 1), which strongly suggested a blockade of Fc receptors interfering with antibody penetration. Pre-incubation of the MNC cells for 1 h with Fc fragments from human IgG at a concentration of 120 μ g per 10⁶ cells, obtained by papain cleavage of the anti-RNP IgG by a modification of the Porter's method¹⁸, resulted in an 80% decrease of intranuclear fluorescence. Fluorescein labelled F(ab')₂ fragments of the anti-RNP IgG prepared by pepsin digestion¹⁸ failed to penetrate into MNC although they remained capable of giving direct nuclear immunofluorescence in frozen sections of rat stomach.

Because of their large size, antibody coated chicken erythrocytes did not block all Fc receptors and allowed penetration of antibody in experiments combining fluorescent labelling and Fc rosette formation. The dose dependent blocking by aggregated IgG and by Fc fragments supports this explanation.

It is apparent that the anti-RNP IgG antibody penetrates viable human MNC via their Fc receptors and reaches the nucleus where its antigen resides. The significance of these findings may be manifold. (1) The potential for antibodies to penetrate living mammalian cells—this antibody cell penetration could have several implications in immunobiology: selective deletion of the

Table 1 Location of fluorescence in mononuclear cells after incubation with isothiocyanate-labelled anti-RNP IgG antibody in various conditions

Condition	Site of fluorescence, % viable cells [§]		
	Nuclear	Membrane	Cytoplasmic
MNC (whole); preincubation with anti-RNP IgG, time:			
5 min (2)*†	0.5±0.7	1.5±2.1	0
15 min (2)	2.5±0.7	2.0±1.4	2.5±2.1
25 min (2)	5.5±2.1	4.0±1.4	3.0±1.4
40 min (2)	11.5±2.1	16.0±1.4	3.0±1.4
50 min (2)	18.5±2.1	19.0±2.8	3.0±1.4
60 min (5)	19.35±2.8	17.6±6.55	5.8±3.4
120 min (2)	19.5±2.1	17.0±2.8	4.0±2.8
180 min (2)	18.5±2.1	18.0±2.8	4.5±2.1
MNC adherent to glass wool (5)‡§ (83.8±3.8% positive for myeloperoxidase staining)	4.7±1.25	1.2±0.8	1.2±1.2
MNC non-adherent to glass wool (5) (6.6±1.1% positive for myeloperoxidase staining)			
B-cell enriched MNC (3)	13.8±2.5	17.8±2.9	5.3±1.7
T-cell enriched MNC (3)	22.2±4.9	7.7±0.6	9.0±2.6
Fc rosette forming MNC (3)	1.4±0.5	13.0±1.4	3.4±1.3
Non-Fc rosette forming MNC (3)	76.5±9.2	0.3±0.6	1.0±1.0
MNC pre-incubated with heat-aggregated Cohn's fraction II (μ g per 10^6 cells)	1.0±1.0	12.3±2.1	1.3±1.15
16 (3)	14.0±3.5	12.3±4.5	9.7±3.2
32 (3)	5.0±2.0	7.0±4.35	5.7±4.0
64 (3)	1.0±1.0	8.3±3.2	0.66±0.6
260 (3)	0.3±0.6	10.7±3.5	0.3±0.6
MNC pre-incubated with Fc fragment of IgG (μ g per 10^6 cells)			
60 (2)	10.5±2.1	9.5±0.7	10.0±5.65
120 (2)	4.5±2.1	14.5±6.4	4.5±0.7
MNC incubated with fluorescein labelled F(ab') ₂ (3)	0	6.0±2.6	0

*Parentheses indicate no. of experiments.

†MNC obtained in Ficoll-Hypaque gradient were adjusted to a concentration of 1×10^6 cells per 0.1 ml of minimum essential medium (Eagle) enriched with 0.8% L-glutamine (MEM) were placed in 0.1 ml aliquots and incubated at 37 °C in a 5% CO₂ and 100% humidity atmosphere. Cells were then washed in phosphate buffered saline pH 7.4 (PBS), resuspended in PBS, incubated 5 min with 0.1 ml of a 0.2% Trypan blue dye solution at room temperature, and counted immediately under epifluorescent (Zeiss: Filters UG 1/3, FT: 420, LP 418), and normal illumination. To determine the percentages of cells showing fluorescence at various sites we consider only those cells found to exclude the Trypan blue dye.

‡In this and all subsequent experiments, the incubation time of the MNC with the anti-RNP antibody or its fractions was 60 min.

§Glass wool obtained from J. T. Baker, Phillipsburgh, N. J., USA, Lot 39,284, was loosely packed into a Pasteur pipette filled with MEM and incubated at 37 °C for 30 min. MNC resuspended in the same medium were carefully layered on top of the glass wool and incubated 30 min. with the pipette in vertical position and the tip closed. The pipette tip was opened and 10 ml of MEM were passed through the pipette to collect non-adherent cells. The pipette was then broken and the glass wool washed in PBS, to obtain adherent cells.

||Mean \pm 1 σ

antibodies, selective triggering of cell proliferation, a mechanism for memory storing, or, if such be the case, interaction with intracellular antigens. (2) A previously unsuspected role for the Fc receptor in the cellular penetration of antibodies. (3) By a peculiar tropism and/or attraction this antibody, once inside the cell, might make its way to the nucleus to interact with its antigen. And (4), a possible new mechanism of immunologically mediated damage might occur in diseases where antibodies to cell constituents, to nucleic acids and to nucleic acid-protein antigens appear and might modify function when entering a living cell. This mechanism would resemble that of stimulation (type V) as proposed in the Roitt's modification of the Gell and Coombs classification¹⁹.

We have found that intranuclear penetration by antibody occurs *in vivo* in human disease. Patients with MCTD have intranuclear immunoglobulin in their circulating MNC, detectable by means of indirect immunofluorescence using goat antibody to human immunoglobulins, which is thus, also capable of penetrating viable cells. Details of this finding will be reported elsewhere.

We thank the Fondo de Fomento Educativo, Mexico, for financial support.

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Received 9 August; accepted 2 November 1977.

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Carcinogen-induced chromosome breakage in Fanconi's anaemia heterozygous cells

FANCONI'S anaemia (FA) is one of the genetic syndromes in which chromosomal instability has been associated with an increased predisposition to cancer¹. An increased risk of malignancy has also been reported for presumptive carriers of the FA gene^{2,3}, although no evidence of chromosome abnormalities has been found in cultured lymphocytes or fibroblasts from the FA heterozygote. The nature of the genetic defect in FA is still unclear, although there is evidence for defective DNA repair⁴⁻⁶.

Table 1 DEB-induced chromosome breakage in Fanconi's anaemia heterozygous and normal cells

Cell strain	Passage no.	Chromatid breaks	Chromosome breaks	Fragments + deletions	Rearrangements*	No. of breaks per cell†
FA heterozygotes‡						
C13						
Untreated	8	3	0	1	0	0.04
Treated	8	7	7	3	2	0.21
C59						
Untreated	12	0	0	0	0	0
Treated	12	8	1	3	7	0.26
C57						
Untreated	12	6	0	0	0	0.06
Treated	12	10	2	9	2	0.25
Normals‡						
C40						
Untreated	12	2	2	2	1	0.08
Treated	12	5	0	0	1	0.07
C39						
Untreated	11	2	0	1	0	0.03
Treated	11	2	0	3	0	0.05

*Rearrangements include translocations, dicentric, and rings and are scored as two breaks.

†100 cells were analysed in each set.

‡FA heterozygous and normal cultures were not identified until after data were tabulated. C13, 31-yr-old male; father of FA patient C12, previously tested using this same *in vitro* system¹⁰; C59, 40-yr-old male; C57, 49-yr-old female; C40, 23-yr-old male; C39, 26-yr-old male.

Lymphocytes from FA homozygotes have an increased incidence of chromosome aberrations after exposure to ionising radiation⁷ or alkylating agents^{8,9}. We reported recently that baseline breakage rates for FA cell strains varied from 0.20 to 0.36 breaks per cell, and that after carcinogen treatment of the type described below, these rates were increased three to fivefold¹⁰. The same carcinogen treatment had no clastogenic effect on normal, xeroderma pigmentosum and trisomy 18 fibroblasts. There is at present no way to distinguish FA heterozygous individuals from normal. The only distinction that has been reported has been an increased susceptibility to *in vitro* transformation by the oncogenic virus SV40 in both homozygous and heterozygous FA fibroblasts¹¹, but similar results have been obtained with cell strains from patients with several other syndromes. As the ability to identify carriers of the FA gene would be of great benefit because of their increased cancer risk, we report here the results of experiments in which we were able to distinguish FA heterozygous cell strains from normals, after exposing the cells to the difunctional alkylating agent diepoxybutane (DEB), an active mutagen and carcinogen¹²⁻¹⁵.

Early passage skin fibroblasts from five individuals were obtained from Dr M. Swift (University of North Carolina at Chapel Hill). We received the flasks coded, and the identity of the strains was revealed only after results of the experiments were tabulated. Cells from each strain were exposed to 0.01 μ g of DEB per ml medium for 6 d, while replicate cultures served as untreated controls. At this concentration, DEB had no cytotoxicity in these strains; treated cells showed approximately the same growth curve as untreated.

The effect of DEB treatment on chromosome breakage in these cells is shown in Table 1. One hundred cells were analysed in each group. The approximately fivefold increase in breakage in the FA heterozygous cells after treatment with DEB was highly significant ($P < 0.001$). The breakage was not concentrated in a few cells, but fit a Poisson distribution. Qualitatively, the chromosomal aberrations induced by DEB in FA heterozygous cells were similar to those which occur spontaneously in FA homozygous cells; open chromatid breaks were the most common finding. In contrast to this result in the FA heterozygous cells, there was no increase in chromosome breakage in normal cells, even those with the highest intrinsic break rate (normal cell strain C40, see Table 1).

Our results indicate that the use of a clastogenic agent in an *in vitro* stress system may have value as a method for determining whether presumptive heterozygotes are in fact gene carriers for FA. In earlier studies exposure of FA heterozygous cells to alkylating agents did not induce increased chromosomal aber-

rations, or increased sister chromatid exchanges greater than that observed in normal cells^{5,8}. Those studies involved direct analysis of exposed lymphocytes. The fibroblast system used in this study may offer a more sensitive means of detecting susceptibility to induced chromosome breakage. Chronic exposure to a non-toxic concentration of the alkylating agent may produce a greater load of DNA damage in a cell population still capable of mitotic division. Perhaps FA heterozygous cells have a reduced capacity to repair DNA, which is not apparent except under the stress of increased damage. As in xeroderma pigmentosum cells^{16,17}, increased sensitivity to chemically-induced chromosome instability occurred in cells lacking spontaneous chromosome breakage.

Although individuals affected with FA are rare, the heterozygote frequency is estimated at 1 in 300 and FA heterozygotes could comprise 1% of all persons dying from cancer². The ability to identify FA heterozygotes would make possible a direct assessment of the risk for carriers of the FA gene acquiring cancer, as compared to the general population. It would also be possible to offer a screening programme for early detection of cancer to individuals identified as FA heterozygotes. The use of an *in vitro* system based on extended exposure to low levels of a carcinogen may provide an effective stress test for the detection of other genes which predispose to cancer.

We thank Drs J. Mitra and R. S. K. Chaganti for helpful discussions, Dr M. Swift for cell strains, and Dr B. M. Goldschmidt for providing the DEB. This study was supported in part by the Martha and Regina Brand Foundation.

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Increased CTP synthetase activity in cancer cells

AN insight into the biochemical strategy of cancer cells has been achieved by the conceptual and experimental approach provided by the molecular correlation concept^{1,2}. Studies carried out using this approach with model systems of rat hepatomas and kidney tumours of different growth rates revealed a biochemical imbalance in cancer cells, which manifested itself in progressive changes in activities of key enzymes and overall metabolic pathways that correlated with tumour growth rate^{1,2}. Some of these biochemical alterations have been shown to apply to human primary liver³ and kidney⁴ carcinomas. Those alterations in gene expression that are linked to the increase in the expression of malignancy are manifested in the increased activities of key glycolytic-, purine-, pyrimidine-, DNA- and polyamine-synthesising enzymes¹⁻⁸. Concurrently, decreases occur in the activities of the key enzymes of gluconeogenesis, purine and pyrimidine catabolism and of the urea cycle^{1-5,9,10}. In addition to such a progression-linked (growth-rate-linked) metabolic imbalance, the reprogramming of gene expression in cancer cells entails transformation-linked alterations that are present in all hepatomas irrespective of growth rate or extent of differentiation^{1,2,11-15}. Here we report that CTP synthetase (UTP:L-glutamine amidoligase, EC 6.3.4.2) increased in all the hepatomas examined, the activity being highest in the rapidly growing tumours. Thus, in liver neoplasia the activity of this enzyme is both transformation- and progression-linked. CTP synthetase activity was also markedly increased in transplantable kidney tumours in the rat and in primary renal cell carcinomas in man.

Our studies on CTP synthetase, the enzyme that catalyses the synthesis of CTP from UTP, were undertaken because the activity of UDP kinase, an enzyme that produces UTP, was elevated in all hepatomas examined¹⁵. In rapidly growing hepatoma 3924A, however, the concentration of UTP was unaltered, but the CTP concentration was increased fivefold¹⁶. An accumulation of CTP might occur because of decreased utilisation, which is not likely in the rapidly growing tumour; or it might be caused by an increase in the activity of CTP synthetase, an enzyme that is a sensitive target for anti-tumour active glutamine antagonists¹⁷. We have tested the hypothesis that the observed increase in CTP concentration may be accounted for, at least in part, by an increase in CTP synthetase activity. Our systematic studies revealed that CTP synthetase is the rate-limiting enzyme in hepatic CTP biosynthesis (Fig. 1). The specific activity of CTP synthetase was increased in all hepatomas examined, and the elevation correlated with the increase in tumour growth rate. This increased potential for CTP biosynthesis provides one mechanism by which the cancer cell can adjust the availability of CTP for RNA synthesis and for the formation of 2'-deoxy CTP for the synthesis of DNA.

Male ACI/N and Buffalo inbred strains of rats and Wistar rats were maintained as described previously³. The procedures for obtaining tumours and regenerating livers have been described³. The growth rates of the various hepatoma lines ranged from 2-52 weeks, as measured by the amount of time required for the neoplasms to reach a diameter of 1.5 cm.

The kinetic characteristics of CTP synthetase in crude enzyme preparations and in partially (50-fold) purified extracts of rat liver and rapidly growing hepatoma 3924A were similar. The pH optima were broad, ranging from 7.0-9.0. The apparent K_m values for the ligands UTP, ATP, glutamine and GTP at pH 7.4 and 37 °C were 0.3, 0.6, 0.1, and 0.07 mM, respectively, both in liver and hepatoma 3924A crude extracts.

The specific activity of CTP synthetase in the liver of freely fed rats ranged from 4-6 nmol per h per mg protein. A comparison of

the activities of enzymes involved in the *de novo* synthesis, salvage or recycling of UMP, and in the conversion of UMP to CTP (Fig. 1), shows that CTP synthetase activity is the lowest^{15,18,19}.

In the liver tumours of slow and medium growth rate the specific activity of CTP synthetase was increased 1.8- to 3.6-fold that of normal rat livers, whereas the activity was increased from 4.6- to 11.2-fold in rapidly growing hepatomas (Table 1).

In 24-h regenerating livers, CTP synthetase activity increased twofold that observed in the liver of sham-operated control rats. This agrees with observations indicating an increase in uridylate biosynthesis in regenerating liver²⁰. CTP synthetase activity in the average liver cell of the 5-d-old rat was 31% of that of normal adult rat. Since increased CTP synthetase activity was present in the hepatomas, but not in the rapidly growing differentiating liver, CTP synthetase activity is not linked with differentiation. Since the regenerating liver has a proliferation rate similar to that of rapidly growing hepatoma 3924A (ref. 1), the elevated activity in this hepatoma (sevenfold) was higher than that in regenerating liver (twofold).

Studies in this laboratory demonstrate a marked step-up in hepatoma CTP biosynthetic capacity^{15,18}. The key enzymes participating in the *de novo* biosynthesis of UMP were increased in parallel with the increase in CTP synthetase activity and with tumour growth rate¹⁸. The salvage enzymes, uridine phosphorylase, uridine kinase, and uracil phosphoribosyltransferase, were similarly elevated¹⁹. UDP kinase was increased in all tumours irrespective of growth rate¹⁵. In contrast, the activity of the rate-limiting catabolic enzyme, dihydrouacil dehydrogenase, was decreased⁹. Thus, the programme displayed in the cancer cells provides for an increased capacity for CTP biosynthesis and a decreased capability for the catabolism of the precursors. These alterations should confer selective advantages on the cancer cells.

Note that CTP synthetase activity was also increased in slowly growing rat kidney tumour MK-3 (3.5-fold) and in three cases of human renal cell carcinoma (2.5-fold).

Our demonstration of high CTP synthetase activity in hepatomas suggests that the reprogramming of gene expression in malignant transformation and progression is linked with the

Fig. 1 Metabolic pathways for CTP biosynthesis and utilisation. Numbers in parentheses are specific activities of the enzymes (nmol per h per mg protein) at 37 °C and pH 7.4. Further details of the metabolic reactions given in schematic form in this figure are cited in the text^{1,2}. ASP, Aspartate; CP, carbamoylphosphate; OMP, orotidine-5'-monophosphate; UMP, uridine-5'-monophosphate; PRPP, 5-phosphorylribose-1-pyrophosphate; U, uracil; UR, uridine; β -Ala, β -alanine; UDP, uridine-5'-diphosphate; UTP, uridine-5'-triphosphate; CTP, cytidine-5'-triphosphate; ATP, adenosine-5'-triphosphate; GTP, guanosine-5'-triphosphate; dGTP, deoxyguanosine-5'-triphosphate; dATP, deoxyadenosine-5'-triphosphate; RNA, ribonucleic acid; DNA, deoxyribonucleic acid.

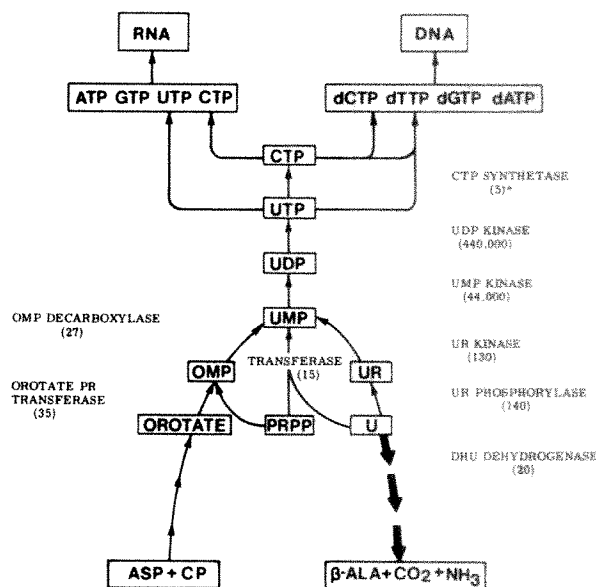


Table 1 CTP synthetase activity in hepatomas of different growth rates

Tissues	Growth rate (months)	Protein		Activity		
		mg g ⁻¹	% Control	nmol per h per mg protein	% Control	
Normal livers from Buffalo rats						
Control for	9618A	100.0±0.6		5.8±0.3		
	28A	111.7±2.0		5.9±0.4		
	8999	100.0±0.6		5.8±0.3		
	7787	111.7±2.0		5.9±0.4		
	44	111.7±2.0		5.9±0.4		
	9633	100.0±0.6		5.8±0.3		
	7777	94.0±1.2		5.1±0.7		
	7288C	98.9±0.9		4.1±0.1		
	9618A2	98.9±0.9		4.1±0.1		
Normal livers from ACI/N rats						
Control for	3924A	95.5±1.2		5.5±0.3		
	3683F	98.4±1.3		5.4±0.2		
Hepatomas						
	9618A	12.4	93.0±4.0	93	19.7±1.4	340*
	28A	6.8	86.9±0.9	78*	21.2±0.6	359*
	8999	6.3	82.2±0.4	82*	10.7±0.4	184*
	7787	6.0	91.7±1.7	82*	11.1±0.5	188*
	44	5.4	98.5±1.5	88*	13.1±0.4	222*
	9633	5.2	76.8±0.8	77*	16.1±0.4	277*
	7777	1.0	59.7±0.7	63*	23.7±1.5	464*
	3924A	0.9	71.0±0.9	74*	38.6±1.4	702*
	7288C	0.8	78.8±0.6	80*	40.7±1.0	999*
	9618A2	0.8	71.7±1.0	72*	37.1±0.8	911*
	3683F	0.5	73.1±0.9	74*	60.6±2.9	1122*

*Significantly different from values of corresponding control liver ($P = <0.05$).

Data are mean \pm s.e. of four or more animals in each group.

Tissue homogenates (15%, w/v) were prepared at 4 °C in 0.15 M KCl, pH 7.4. CTP synthetase activity was determined in the 100,000g supernatant fluids by measuring the conversion of 4-¹⁴C-UTP to 4-¹⁴C-CTP. The components (mM) of the incubation mixture (pH 7.4, 37 °C) were contained in 200 μ l: glycylglycine, 70; MgCl₂, 18; beta-mercaptoethanol, L-glutamine and NaF, 10; phosphoenolpyruvate and ATP, 8; GTP, 1; UTP, 2; 4-¹⁴C-UTP (60 mCi mmol⁻¹), 0.03; and supernatant fluid, 100 μ l. The assay was initiated by addition of supernatant fluid and terminated at 5-min intervals by adding perchloric acid and immersing the reaction tube in an ice-bath. Samples were neutralised with ethylenediamine tetraacetic acid-KOH solution and the potassium perchlorate removed by centrifugation. Ten microlitres of the clear supernatant fluid was applied on PEI-cellulose plates (Brinkmann) together with 20 nmols of a carrier mixture containing the mono-, di- and triphosphates of uridine and cytidine. Ascending chromatography was carried out at 4 °C in rectangular glass tanks which contained 100 ml 0.8 M ammonium sulphate as described previously²¹. CTP was located approximately 2 cm below the UTP spot as determined using ultraviolet light and autoradiography. Ultraviolet absorbing spots were cut out and placed into counting vials with scintillation fluid (Econofluor, New England Nuclear). Radioactivity was determined in a Packard Tri-Carb (Model 3390) liquid scintillation spectrometer. Protein concentrations were assayed²², using recrystallised bovine serum albumin as standard. CTP synthetase activity was linear with amount of enzyme added and time elapsed in the assay; specific activity was expressed as nmol substrate metabolised per h per mg protein.

expression of the CTP-synthesising enzyme activity. In this laboratory, 12 such transformation-linked increases in enzyme activities have so far been discovered, and they all relate to an increased potential in the channelling of metabolites to strategic biosynthetic processes. The increase in the activities of glucose-6-phosphate dehydrogenase (EC 1.1.1.49) and transaldolase (EC 2.2.1.2) provides an increased capacity for channelling glycolytic intermediates into pentose phosphate biosynthesis. Elevations in the activities of phosphoribose-1-pyrophosphate (PRPP) synthetase (EC 2.7.6.1)¹² and glutamine PRPP amidotransferase (EC 2.4.2.14)¹³ provide an increased potential for utilising ribose-5-phosphate and PRPP for *de novo* purine biosynthesis. Increases in the activities of adenylosuccinate synthetase and adenylosuccinase should yield an increased capacity for the *de novo* production of adenine nucleotides³. The elevated activity of AMP deaminase (EC 3.5.4.6)³ and IMP dehydrogenase (EC 1.2.1.14)⁶ provide an increased capability for IMP formation and its utilisation in the *de novo* biosynthesis of guanine nucleotides. The increased activities of uridine kinase (EC 2.7.1.48), uridine phosphorylase (EC 2.4.2.3), uracil phosphoribosyltransferase (EC 2.4.2.9)¹⁹ and UDP kinase¹⁵, provide an increased capacity for UTP biosynthesis. In contrast, the activities of certain catabolic enzymes are decreased in the hepatomas, including inosine phosphorylase (EC 2.4.2.1)¹⁹, xanthine oxidase (EC 1.2.3.2), uricase (EC 1.7.3.3) and thymidine phosphorylase (EC 2.4.2.4)¹⁴. The display of this transformation-linked programme indicates that an enzymatic imbalance emerged in neoplasia reflecting ordered and reciprocal alterations in gene expression. Some of these enzymes constitute promising points for attack in

the design of selective enzyme-pattern-targeted chemotherapeutic agents and offer opportunities for selectively preventing host toxicity by means of metabolite rescue²³.

This work was supported by the USPHS.

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Central nervous system effects of limb amputation in man

WE report here the results of an electrophysiological study of the central effects of long-standing limb amputation in man. The findings are felt to have significance for an understanding of neurotrophic mechanisms. The results show that, after amputation, the impulse-conducting ability of a peripheral nerve is severely reduced and that this is accompanied by significant changes in the evoked cortical response; in contrast, function in certain reflex pathways is apparently well maintained. This type of study complements a number of early anatomical investigations^{1,2}. In reviewing the early studies it is evident that much disagreement exists concerning the extent of the atrophy to be found in the cells of the anterior and posterior horns, and in the myelinated fibres of the anterior and posterior roots and of the ascending and descending tracts in the spinal cord. There is the added problem, common to all neuropathological studies, of deciding to what extent impulse conduction and synaptic transmission might have been interfered with. By using a functional approach our study has resolved some of these uncertainties.

The study was performed on 10 male patients, aged 11 to 47 yr. Nine patients had suffered amputation of a hand and part of the forearm and in one case a similar loss was the result of a congenital anomaly; the durations of the lesions varied from 4 months to 30 yr. In each patient the central stump of the ulnar nerve was stimulated maximally below the elbow and the compound action potential was recorded between the elbow and axilla. Recordings were also made from the somatosensory 'hand' area of the contralateral cortex using conventional techniques^{3,4}. Finally, the reflex effects of such stimulation were studied on volitional activity in the ipsilateral triceps muscle using rectification and averaging⁵. All the stimulating and recording procedures were performed with surface electrodes; these comprised chlorided silver disks or cups coated with a conducting cream. Control observations were made, using similar electrode placements, on the intact arms of the patients and on both arms (and contralateral somatosensory cortices) of 15 healthy controls. All the patients and control subjects had given informed consent for the procedures.

The results in the amputees were consistently abnormal in several respects (Fig. 1 and Table 1). First, the compound action potential in the ulnar nerve was markedly reduced on the side of the amputation, the mean peak-to-peak amplitude being only 13% of that on the control side. Maximum impulse velocities in the conducting fibres were usually normal, though low velocities in two subjects caused the mean value to be

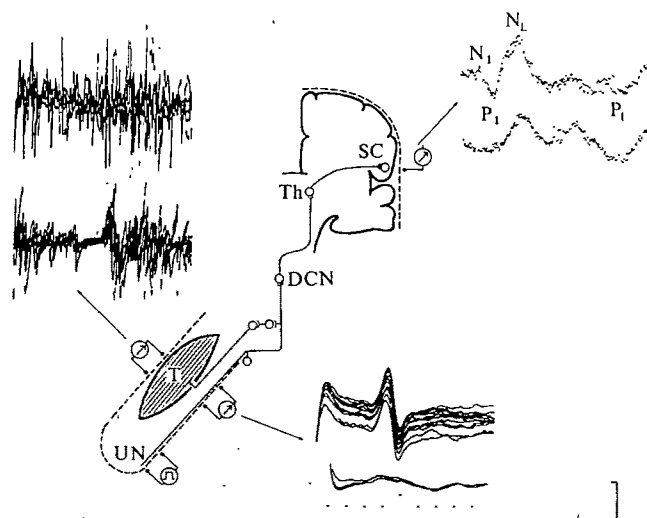


Fig. 1 Experimental arrangement, neural pathways, and results of study in a 32-yr-old man who had suffered traumatic amputation of his right hand 3 yr previously. In each pair of recordings the upper and lower traces show the results of stimulating ulnar nerves (UN) in the intact and partially-amputated arms respectively. Stimuli were delivered at the start of each trace. Recordings from somatosensory cortex (SC) were averaged 32 times in each of two trials. The recordings from the triceps muscle (T) were made during weak voluntary contraction and several traces have been superimposed (rectified and averaged responses not shown). Vertical bar represents 10 μ V, 20 μ V and 40 μ V for recordings from cortex, peripheral nerve and triceps respectively; horizontal bar represents 40 ms except for peripheral nerve traces in which calibration marks denote milliseconds. DCN, dorsal column nuclei; Th, thalamus; triceps reflex pathway simplified.

significantly smaller. Second, the earliest negative (N_1) and positive (P_1) components of the evoked cortical responses were significantly reduced, being undetectable in four patients; in contrast, later components (N_L - P_L in Table 1) were better preserved. Finally, volitional activity in the triceps muscle was modulated to a greater extent by inputs from the amputated ulnar nerve stump than from the nerve on the intact side; this applied both to the initial inhibitory phase (40-100 ms post-stimulus) and to the succeeding excitatory phase. Control observations in normal subjects suggested that the reason for the discrepancy was that inhibition had decreased on the intact side of the amputees, presumably as a result of the extra use made of that limb after injury to the opposite arm.

These results suggest that, in normal circumstances, the functional integrity of motor and sensory axons in human limb nerves is heavily dependent on intact connections with muscle fibres and receptor tissues. Despite the severe loss of excitable axons which follows amputation, sufficient sensory nerve fibres can remain functional to elicit normal polysynaptic reflexes (involving ipsilateral motoneurons with intact axons). Finally, it is probable that there is some loss of function in the

Table 1 Comparison of responses evoked in ulnar nerve, ipsilateral triceps muscle, and contralateral somatosensory cortex following maximal stimulation of ulnar nerves in partially-amputated and intact arms of patients

		Patient limbs			P
		Control limbs (30)	Intact (10)	Amputated (10)	
Nerve	Action potential (μ V)	71.1 \pm 26.8	66.1 \pm 34.1	8.7 \pm 8.6	<0.001
	Conduction velocity (m s ⁻¹)	76.9 \pm 7.6	73.6 \pm 8.6	68.2 \pm 9.6	<0.02
Cortex	N_1 - P_1 response (μ V)	3.9 \pm 1.8	4.3 \pm 1.2	1.4 \pm 1.4	<0.001
	N_L - P_L response (μ V)	8.8 \pm 2.8	9.4 \pm 3.8	6.2 \pm 2.5	<0.01
Triceps	Inhibition (%)	75.4 \pm 17.0	41.8 \pm 25.7	79.7 \pm 25.9	>0.5
	Excitation (%)	46.6 \pm 26.0	42.8 \pm 20.9	68.4 \pm 51.1	>0.2

Results in 15 normal subjects are shown for comparison. Parentheses indicate numbers of limbs studied. Significance levels calculated by Student's *t*-test and refer to differences between results of nerve stimulation in partially-amputated limbs and in arms of control subjects.

dorsal column–medial lemniscus pathway subserving the early components of the evoked cortical response, beyond that due to lesions of the peripheral sensory nerve fibres. Further studies, aimed at documenting the time course of the central effects or at preventing their occurrence, would be of considerable interest.

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Received 19 August; accepted 4 November 1977.

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Acetylcholine-induced channels and transmitter release at human endplates

MYASTHENIA gravis, a disease affecting the human neuromuscular junction, is thought to result from a postsynaptic defect, namely a reduction in the number of functional acetylcholine (ACh) receptors (reviewed in ref. 1). Thus at myasthenic endplates the spontaneous miniature endplate potentials (m.e.p.ps) and impulse-evoked endplate potentials (e.p.ps) are reduced in amplitude². Furthermore, the diminished number of α -bungarotoxin binding sites^{3,4} indicates a reduction in the number of ACh receptors in the postsynaptic membrane which leads to a reduced sensitivity to ACh⁵; and preliminary experiments with voltage noise (B. Katz, R. M. & J. Newsom-Davis, unpublished) suggested that the decrease in sensitivity does not involve changes in the characteristics of the channels. We have further examined the properties of channels opened by ACh at normal and myasthenic endplates as well as some characteristics of transmitter release in the two conditions. The experiments described here indicate that the single channel conductance and the mean channel life-time are similar in normal and myasthenic muscle membranes but that there are differences in the Ca-dependence of transmitter release from normal and myasthenic nerve terminals.

Intercostal muscle from myasthenic and control patients were dissected to give a small bundle of muscle fibres, sometimes with its nerve supply. Stimulation of the nerve was via a glass capillary suction electrode. Preparations were perfused with an oxygenated medium and examined using conventional electrophysiological techniques for intracellular recording and voltage clamping. For noise experiments, ACh was applied iontophoretically from a micropipette (25–50 M Ω) positioned between the recording and voltage-clamping microelectrodes.

To study the characteristics of channels induced by ACh we have investigated miniature endplate currents, which result from the action of single packets of transmitter on the muscle membrane, and also the membrane current noise which occurs during the application of ACh⁶. As at the nerve–muscle junctions of other species⁷, m.e.p.cs at voltage-clamped human endplates vary greatly in amplitude and shape, with some m.e.p.cs being clearly composite. The decay of the faster m.e.p.cs was exponential^{8–10}, however, and these were used for subsequent analysis.

Examples of a normal and a myasthenic miniature endplate current are illustrated in Fig. 1 ($V_m = -80$ mV, $T = 23^\circ\text{C}$) which shows a semi-logarithmic plot of their decay as a function of time. Comparison of the time constant of decay of m.e.p.cs ($\tau_{\text{m.e.p.c.}}$) from normal and myasthenic endplates reveals that $\tau_{\text{m.e.p.c.}}$ is very similar in the two situations; $\tau_{\text{m.e.p.c.}}$ (normal) = 2.0 ± 0.1 ms (mean \pm s.e.), $\tau_{\text{m.e.p.c.}}$ (myasthenic) = 2.1 ± 0.1 ms. At both normal and myasthenic endplates the duration of m.e.p.cs was prolonged by hyperpolarisation of the membrane, as occurs in frog and toad muscle^{8–10}.

The correspondence of the values for the duration of $\tau_{\text{m.e.p.c.}}$ at myasthenic and normal endplates may be taken to reflect a

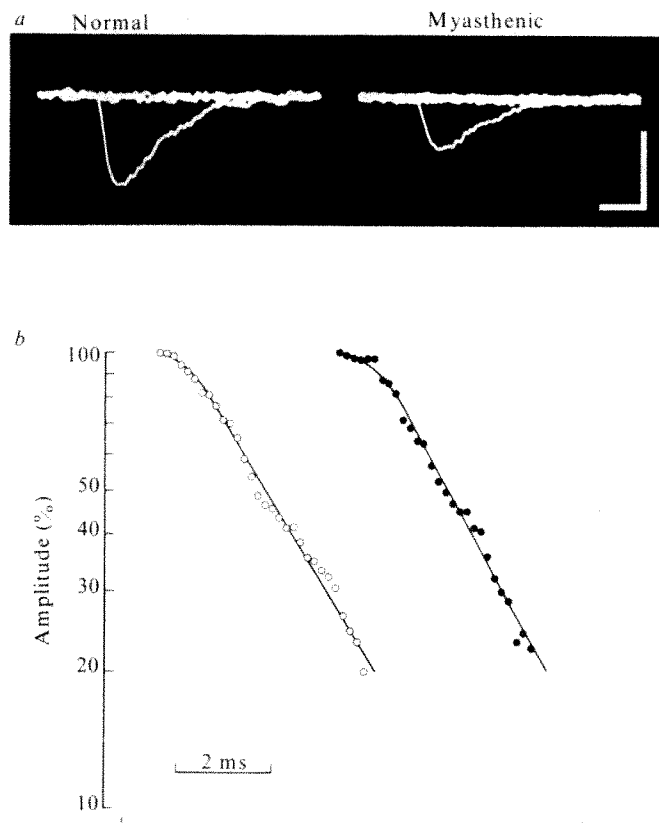


Fig. 1 *a*, Typical miniature endplate currents from normal and myasthenic human endplates. The figures are of several superimposed traces replayed from analog tape. M.e.p.cs were recorded under voltage clamp ($V_m = -80$ mV, $T = 23^\circ\text{C}$) through a 500-Hz low-pass filter which prolongs the rising phase and slightly reduces the amplitude but does not greatly affect the time constant of decay. Calibration 2 ms and 2 nA. *b*, Decay phases of the m.e.p.cs shown in *a*, plotted on semi-logarithmic co-ordinates. Straight lines through the points were fitted by the least squares method to values between 80–20% of the m.e.p.c. amplitude to give the time constant of decay. \circ , Normal $\tau_{\text{m.e.p.c.}} = 1.9$ ms; \bullet , myasthenic $\tau_{\text{m.e.p.c.}} = 1.8$ ms.

similarity in the duration of the mean open state of the acetylcholine channel in the myasthenic and normal muscle membrane, providing other factors, such as time for diffusion of acetylcholine from the cleft¹¹, are equal. It has been possible to derive certain of the properties of the single endplate channel by analysing the voltage-clamped noise which occurred during the steady iontophoretic application of ACh to endplates. In similar experimental conditions, iontophoretic application of ACh produced consistently smaller mean currents at myasthenic than at normal endplates, indicating a reduced postsynaptic sensitivity to ACh.

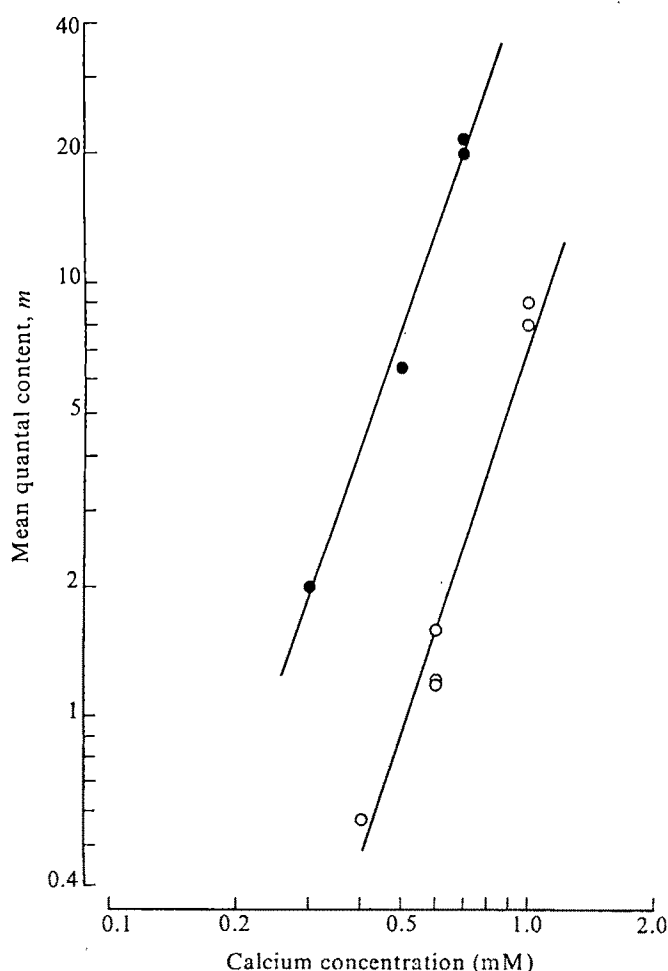
The mean life-time of the channel (τ_{noise}) was obtained^{6,12} from the cutoff frequency (f_c) of the power spectrum (the frequency at which the spectrum has decreased to half of the zero frequency asymptote) by fitting a single Lorentzian component of the form $S(f) = S(0)/(1 + (f/f_c)^2)$ to the spectrum, where $S(f)$ and $S(0)$ are the spectral densities at frequency f and 0 Hz respectively; hence $\tau_{\text{noise}} = 1/(2\pi f_c)$ (ms). The single channel conductance, γ , was obtained from both the variance of the current noise and the zero frequency asymptote of the spectra ($\gamma = \sigma^2/\mu_1(V_m - V_{\text{eq}})$ and $\gamma = S(0)/2\mu_1\tau(V_m - V_{\text{eq}})$, where μ_1 = mean membrane current, V_m = membrane potential, V_{eq} = equilibrium potential for ACh, σ^2 = variance of membrane current fluctuations). In estimating the conductance of the single channel, the equilibrium potential for the endplate current at the human endplate was taken as zero mV (based on unpublished observations).

The mean open time of the ACh-induced channel, obtained by analysis of current noise spectra for normal endplates was, τ_{noise} (normal) = 1.54 ± 0.05 ms (mean \pm s.e.). The comparable value for myasthenic endplates was, τ_{noise} (myasthenic) = 1.63 ± 0.1 ms, at $V_m = -80$ mV and $T = 23^\circ\text{C}$. In addition, the single channel conductance, γ , for normal and myasthenic endplates fell within

the range 20–25 pS. The similarity between the channel characteristics at normal and myasthenic endplates indicates that the properties of ACh-induced channels in myasthenia gravis are virtually unchanged.

As would be expected from earlier work on other mammals, the mean number of transmitter quanta, m , released per nerve impulse from normal and myasthenic nerve-terminals, depends on the extracellular Ca concentration. Figure 2 shows the mean quantal content of e.p.ps, obtained at typical normal and myasthenic endplates; over a range of Ca concentrations. In both cases, m increased as a function of Ca with a slope of approximately 3 on logarithmic co-ordinates. This compares with the value of approximately 3 for rat^{13,14} and for the mouse nerve-muscle junction¹⁵. Although the relation between transmitter release and external Ca concentration had the same slope value for normal and myasthenic nerve terminals, the relationship at myasthenic endplates was shifted to the left when compared with normal. Thus at any given Ca concentration in the range studied (0.3–1.0 mM) more quanta per impulse were released from myasthenic than from normal terminals. For the results from the two endplates illustrated in Fig. 1, the myasthenic terminals released approximately 8 times more quanta per impulse than the normal terminals. The size of this ratio varied from endplate to endplate in different muscles, the mean ratio being around 5:1 (myasthenic: normal) at 26 myasthenic and 12 normal endplates

Fig. 2 Relationship between extracellular calcium concentration and the mean quantal content, m , for a myasthenic endplate (●) and a control endplate (○). Each point represents the average response to 30–180 impulses. Lines with slopes of 3 have been fitted by eye through the points. At any given concentration the myasthenic nerve terminals release approximately eight times more transmitter packets. For the myasthenic curve, m is estimated indirectly from mean e.p.p.²/e.p.p. variance; for the normal curve, m is estimated from mean e.p.p./mean m.e.p.p. at each Ca concentration; additional values at 0.6 and 1.0 mM Ca were estimated from mean e.p.p.²/e.p.p. variance or from \ln (no. of impulses/no. of failures).



investigated over the range of Ca concentrations 0.25–0.7 mM (2 mM Mg). The size of the ratio became less at Ca levels greater than 0.7 mM. In the presence of 2 mM Ca (and 2 mM Mg), myasthenic terminals seem to release about twice as many quanta per nerve impulse as normal terminals. A number of possible factors can be envisaged to account for the higher levels of transmitter release from myasthenic nerve terminals. Briefly, these include: an increase in the synaptic area of myasthenic terminals (ref. 16, but see ref. 17); an increase in the level of intracellular free Ca caused either by a greater than normal influx of Ca during the nerve terminal action potential or by a diminished removal of Ca. Further studies should allow us to distinguish between these and other possibilities.

The release of a larger number of transmitter quanta per nerve impulse, from myasthenic terminals when compared with normal, may be related to biochemical studies which have shown that myasthenic muscle contains about twice as much ACh as normal¹⁸. It may be that transmitter release increases, following the impairment of transmission, as a means of 'compensating' for the reduced postsynaptic sensitivity.

We conclude that the reduction in α -bungarotoxin binding sites and in postsynaptic sensitivity at myasthenic endplates results from a loss of functional receptor-channel complexes rather than a modification of individual ionic channels. In addition, the slope value of about 3 for the Ca-dependence of transmitter release at both myasthenic and normal endplates suggests that the mechanisms underlying Ca-dependence may be unchanged. At a given Ca concentration, however, the number of transmitter packets released per nerve impulse is greater for the myasthenic than for the normal nerve terminals.

We thank Mr J. R. Belcher, F.R.C.S., Dr J. Newsom-Davis, Mr M. F. Sturridge, F.R.C.S. and Dr A. Vincent for their co-operation, and the MRC for support. We are grateful to the patients for their collaboration. S.G.C.C. is a Beit Memorial Research Fellow.

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Neural induction of the 16S acetylcholinesterase in muscle cell cultures

SEDIMENTATION analysis of rat muscle acetylcholinesterase (AChE) has revealed the existence of three molecular forms, 4S, 10S and 16S (refs 1, 2). The 16S form was exclusively found in skeletal muscle segments containing neuromuscular junctions; it disappeared after denervation^{1,2} and reappeared following reinnervation either at the original endplate, or at ectopic sites. The 16S AChE may therefore be referred to as an 'endplate specific' form, but it clearly does not represent all activity at the endplate. It took several days for the disappearance of the 16S form after denervation, suggesting a postsynaptic localisation². In posterior leg muscles from rat embryos, the 16S form was absent before the

14th day of gestation; it appeared at day 15 and was relatively more abundant on day 16 (25% of the total AChE activity) than in the adult (5%). (The total AChE activity increased by a factor of 10^3 from day 16 of gestation to the adult muscle².) We present here some data concerning the synthesis of the 16S form by embryonic rat cells in culture, which demonstrate that this form is produced by muscle cell cultures on induction by neuronal elements.

Myoblasts were put in culture after mechanical dissociation of posterior leg muscles, from either 13–14- or from 18-d-old rat embryos. At the earlier age, no 16S AChE could be detected, whereas it was present in the older embryos. Spinal cord cells from

14-d-old embryos were dissociated by gentle trypsinisation or by mechanical techniques, and were plated alone, or added to myoblast cultures. The culture medium was renewed every 3 d for up to 4 weeks, when the cells peeled away from the dishes. The morphology of the cells was examined, and AChE was detected by the histochemical method of Koëlle and Friedenwald³, as modified by Couteaux and Taxi⁴, using acetylthiocholine as substrate. The molecular forms of AChE were analysed, after solubilisation in a buffer containing detergent, by sucrose gradient sedimentation². Their biosynthesis was studied after complete and irreversible inhibition (DFP), by analysing activity during recovery.

Myoblasts from 13–14-d-old embryos possessed only the 4S and 10S forms of AChE, and even after 15 d in culture, it was impossible to detect any 16S form. Similar results were obtained with cultures of spinal cord neurones. When dissociated neurones were added to a myoblast culture (2–3 h after plating, however, we observed the appearance of 16S AChE after 5 d in the mixed cultures (Fig. 1). At this stage, spots of AChE activity were detectable histochemically on the membrane of the myotubes which were coupled with the nerve cells (Fig. 2). Conversely, AChE spots were never detected on myotubes cultivated without nerve cells. In cultures coupled for 10 d, the spots gave place to typical end-plates (J. K., unpublished results). The evolution of the membranous localisation of the AChE activity suggests that the AChE spots observed at 5 d correspond to the foremost neuromuscular contacts. The negative histochemical results of Fischbach *et al.*³ may be due to the fact that the AChE activity of chicken coupled cultures is too weak to be detected by the method they use.

In contrast with 14-d-old myoblasts, muscle cells from 18-d-old embryos contained the 16S form of AChE in addition to the 4S and 10S forms, at the time of plating. The specific activity and the relative proportions of all forms remained stable in the cultures until the cells were peeling from the dishes at 4 weeks. To determine whether this was simply due to the maintenance of AChE molecules, or whether the myotubes were able to synthesise this molecular form, we inhibited all esterase activity in 8-d cultures by treatment with DFP (10^{-5} M for 1 h). Within 48 h, the AChE activity and its distribution in 4S, 10S and 16S forms had recovered. The forms appeared in succession, suggesting that the lighter molecules are precursors of the heavier ones (Fig. 3).

The results presented here clearly demonstrate that the 16S form of AChE may be synthesised by muscle cells, since the contaminating cells of myoblast cultures, fibroblasts and Schwann cells do not contain the 16S form. We never detected this form in cultures of a fibroblast cell line nor in spinal cord cultures which also contain Schwann cells. As mentioned earlier, a muscular

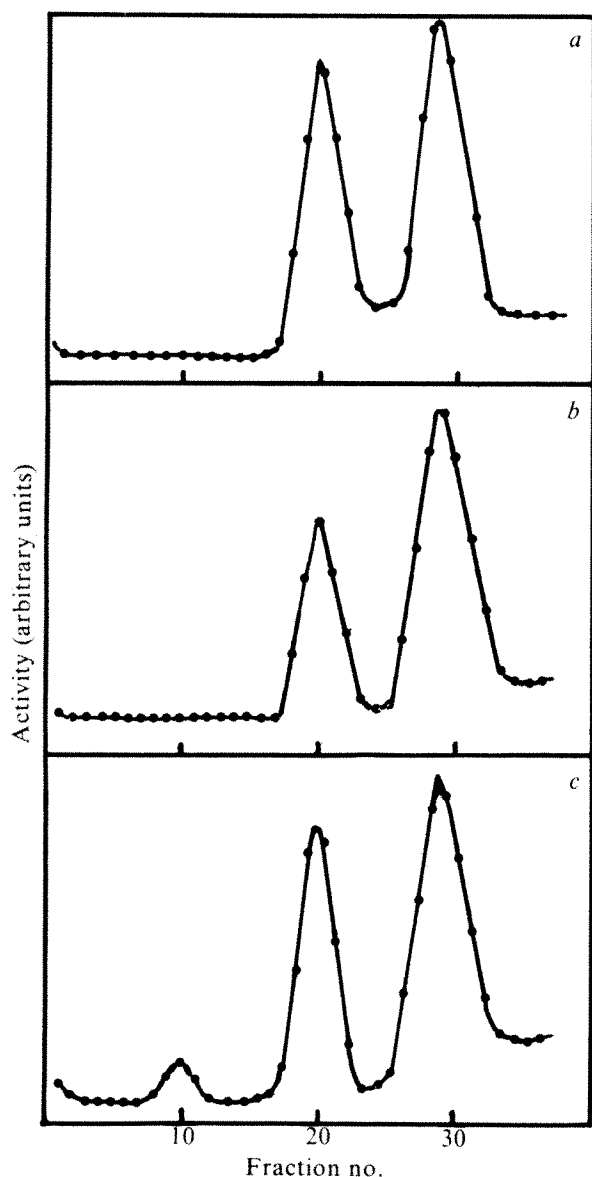
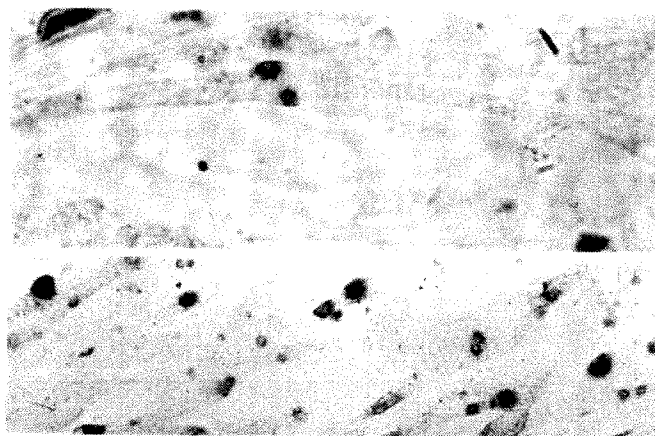


Fig. 1 The molecular forms of acetylcholinesterase in cell cultures of *a*, spinal cord neurones; *b*, myotubes and *c* mixed cultures. 5×10^6 myoblasts or cells from one spinal cord were plated in 50-mm dishes. In mixed cultures, one spinal cord was added to a myoblasts culture 2–3 h later. Cells were grown in 80% MEM, 10% medium 199, 10% horse serum and 0.5% chick embryo extract medium. After 5 d cells were scraped from the dishes, gathered and homogenised using a Potter homogeniser in a solution containing 1 M NaCl, 5×10^{-2} M $MgCl_2$, 10^{-2} M Tris-HCl pH 7 and 1% Triton X-100. The homogenate was centrifuged at 20,000g for 30 min at 4 °C and the supernatant assayed immediately for AChE activity and analysed on a sucrose gradient. AChE activity was determined at 20 °C by the method of Ellman *et al.*⁵. Sedimentation ultracentrifugation on linear sucrose gradients (5–20%) in presence of the homogenising buffer was performed on a Spinco LII 65 ultracentrifuge, using a SW60 rotor at 125,000g for 15 h at 2 °C. Fractions were collected and assayed immediately for enzymatic activities. B-Galactosidase (16S) and horse liver alcohol dehydrogenase (4.8S) were used as enzyme markers. The recovery of AChE activity after sedimentation procedures was higher than 80%.

Fig. 2 Areas of high activity of AChE visualised as 'spots' on the membranes of young myotubes by the histochemical method of Koëlle and Friedenwald⁶ as modified by Couteaux and Taxi⁷. Fixation was in neutral formaldehyde 5% for 5 min, followed by incubation in Koëlle's medium using AChE as substrate at pH 5 for 12 h. Cultures of myoblasts obtained from 13–14-d-old embryos coupled for 5 d with cord cells of 14-d-old embryos. The cell culture conditions were as in Fig. 1 $\times 800$.



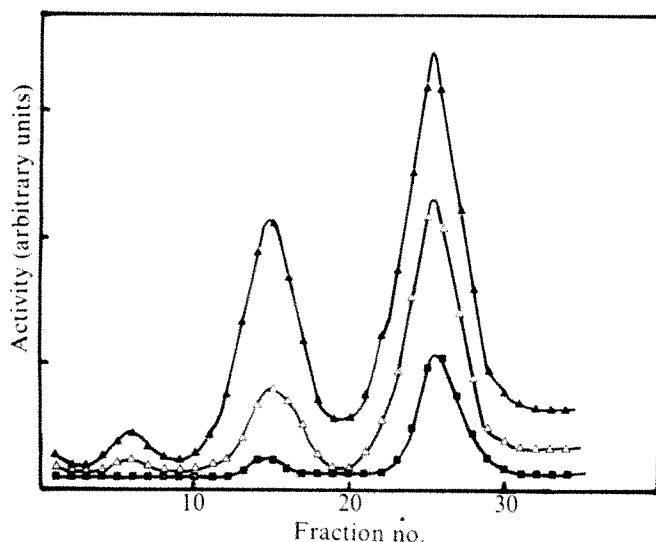


Fig. 3 Restoration of AChE activity after DFP inhibition in cell culture of myotubes from 18-d-old embryos. Here 10⁶ myoblasts were plated in 60-mm dishes. Cells were incubated for 1 h in presence of 10⁻⁵ M DFP in the culture medium to inhibit AChE activity. Such treated cells were washed in order to eliminate excess free DFP and incubated in the culture medium for periods indicated. Other experimental conditions were as in Fig. 1. ■, Cells 7 h after DFP incubation; Δ, cells 14 h after DFP incubation; ▲, cells not incubated with DFP or cells 48 h after DFP incubation.

localisation was implied by the slowness of its disappearance in denervation experiments, but this was not conclusive for its biosynthesis.

From studies of neuroblastoma cell cultures⁴ it has been concluded that the 4S form is a precursor of the 10S form. The present observations also suggest that these forms might in turn be precursors of the 16S molecule.

The most interesting feature of the 16S form of AChE is that its synthesis by muscle cells requires an interaction with neuronal elements, since it only appears in mixed cultures, or in cultures from 18-d-old embryo cells, which have made contact with neurones *in vivo* before being plated. In this second case, the continued synthesis of 16S AChE by these cells, in the absence of neurones, seems paradoxical in view of the disappearance of this form from denervated adult muscles. The experimental situations are, however, so different that it is difficult to interpret this phenomenon and it might well reflect the plasticity of embryonic nerve-muscle interactions.

It would be extremely useful to understand how the 16S molecule is constructed and if non-catalytic elements are included in this complex structure, in addition to knowing how their synthesis and assembly are regulated. The observations reported here not only demonstrate the muscular origin of the 16S acetylcholinesterase and its induction by neuronal elements, but the two types of cultures described provide excellent models for future investigation of the nature of the interaction involved.

We thank Professor G. Buttin and Dr M. Lambiotte for culture facilities and Ms P. Cloup, M. Orouet and D. Dufo for technical assistance. We are grateful to Dr J. Massoulié for helpful discussions and critical comments. This work was supported by funds from the Centre National de la Recherche Scientifique and from the Institut National de la Santé et de la Recherche Médicale.

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Received 28 July; accepted 28 October 1977.

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Effects of [Ca²⁺] and [Mg²⁺] on the decay of miniature endplate currents

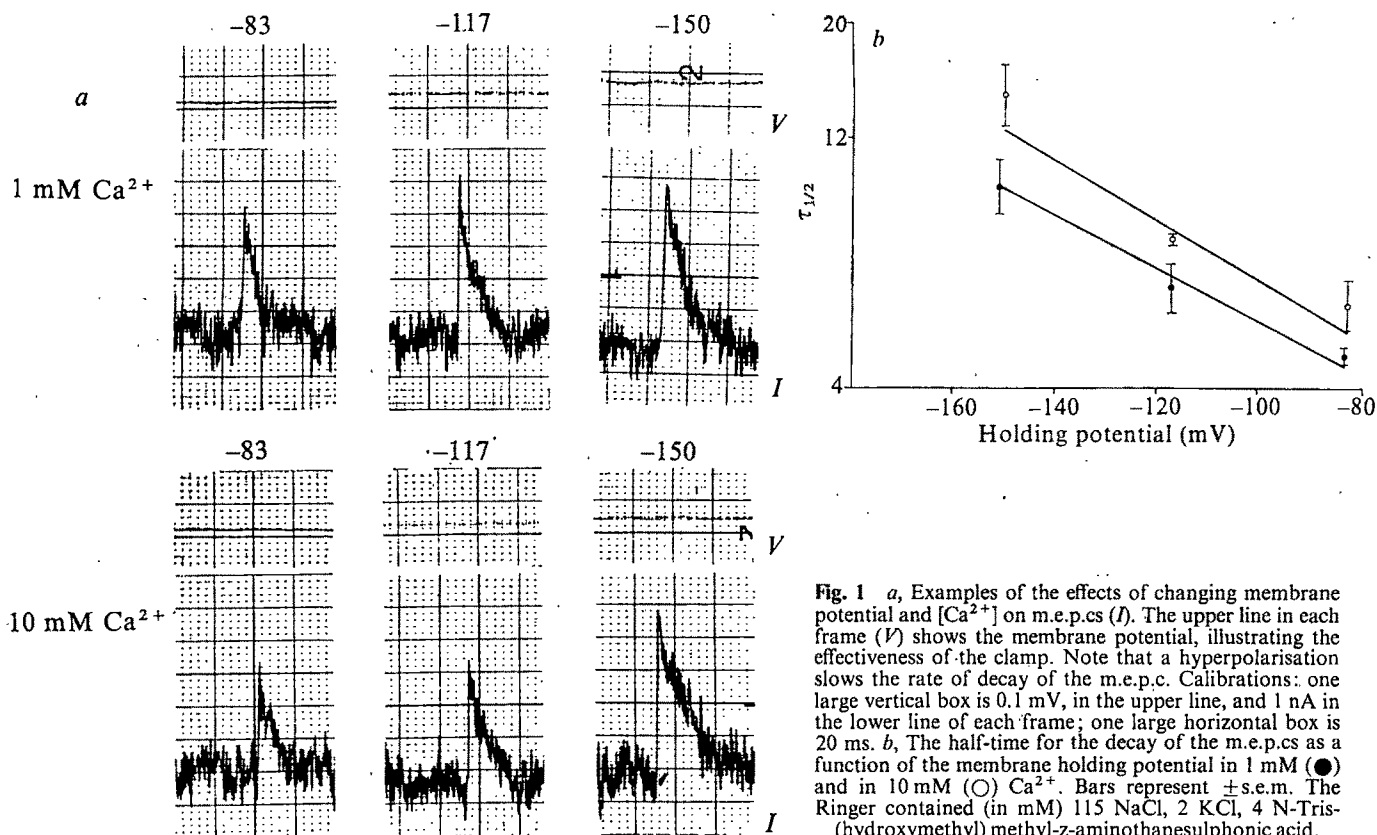
THE endplate current at the neuromuscular junction is initiated by the combination of acetylcholine (ACh) with its receptor. The current rises rapidly to a maximum, and then decays exponentially¹. The rate of decay of the endplate current (e.p.c.) depends on membrane potential; hyperpolarisation lengthens the decay phase^{2,3}. A plausible hypothesis for this is that the rate of disassociation of the ACh from the receptor depends on the voltage gradient through the membrane⁴. The membrane potential consists of three components: the voltage gradient through the membrane itself and the surface potentials at the two membrane-solution interfaces. The surface potentials are thought to be negative, owing to an excess of negatively-charged groups on the membrane phospholipids. An elevation in the divalent ion concentration in the extracellular solutions reduces the negativity of the external surface potential, either by binding to the phospholipids or by screening the charges. A lowering of the negativity of the external surface potential leads to a corresponding increase in the voltage gradient within the membrane (over shorter periods than for phospholipid flip-flop to occur). This leads to the prediction that alterations in the extracellular [Ca²⁺] or [Mg²⁺] are likely to change the time course of the e.p.c. Our experiments were designed to test this hypothesis.

We studied spontaneously occurring miniature endplate currents (m.e.p.cs) to avoid problems caused by raised divalent ion concentrations, such as, changing quantal outputs from stimulated nerves or alterations in the shape of the presynaptic action potential. The two-microelectrode voltage-clamp technique was used at the neuromuscular junction of the sartorius muscle of the frog, *Rana pipiens*¹. The microelectrodes were inserted close to the centre of the junction, as judged by the rise times of the miniature endplate potentials (m.e.p.ps). One electrode monitored the membrane potential, the other passed the clamping current. Both were filled with 4 M potassium acetate. Experiments were carried out both in the presence and absence of 10⁻⁶ g ml⁻¹ neostigmine methylsulphate, which has been shown to slow the decay phase of the m.e.p.cs without altering their voltage dependence⁵.

The results of one experiment in the presence of neostigmine are shown in Fig. 1. The [Ca²⁺] concentration was varied tenfold and m.e.p.cs were recorded between -83 mV and -150 mV. From the recordings, decay half-times ($\tau_{1/2}$) were measured. The tenfold increase in [Ca²⁺] caused roughly the same slowing of the decay as a hyperpolarisation of 20 mV. In six additional experiments, four in the presence of neostigmine and two in its absence, there was a consistent slowing of the decay time course of the m.e.p.cs in elevated [Ca²⁺]. (The magnitude of this effect seemed somewhat smaller in the neostigmine-free preparations.)

To test whether this effect was specific to Ca²⁺ or a more general characteristic of divalent cations, the experiments were repeated varying the Mg²⁺ concentration. One example in the absence of neostigmine is shown in Fig. 2. In this experiment there was a slowing of the decay of the m.e.p.cs equivalent to a 12-mV hyperpolarisation when the [Mg²⁺] was altered from 1 to 10 mM. Similar effects were seen in six additional experiments (two more in the absence of neostigmine and four in its presence).

Since Mg²⁺ and Ca²⁺ produce similar effects in lengthening the decay phase of the m.e.p.cs it seemed possible that the effects might be produced exclusively by the screening of the surface charges by the divalent ions in the solution. The relation between membrane surface charge density, σ (charges cm⁻²), and membrane surface potential, ψ (V), in solutions with different con-



concentrations of univalent, i^+ , and of divalent, i^{2+} , ions (mol cm^{-3}) is

$$\sigma^2 = \frac{\epsilon k T}{2\pi} \left[[i^+] [\exp(Q\psi/kT) + \exp(-Q\psi/kT) - 2] + [i^{2+}] [2\exp(Q\psi/kT) + \exp(-2Q\psi/kT) - 3] \right] \quad (1)$$

where k is the Boltzmann constant, T is temperature in degrees Kelvin, Q is the charge on the electron, and ϵ is the permittivity of water⁵.

The data in Fig. 1 show that a tenfold increase in $[\text{Ca}^{2+}]$ produces an effect that could be attributed to a 20-mV decrease in ψ . By setting up equation (1) for the two divalent ion concentrations, it is possible to solve the pair of simultaneous equations for ψ . In 1 mM $[\text{Ca}^{2+}]$, ψ is about -100 mV, which would be produced by a charge density, σ , of about 1 charge per 93 \AA^2 . Such a high surface potential seems unlikely and in other experiments the change in ψ produced by a tenfold rise in extracellular divalent ion concentration seems to be even greater. Consequently it is unlikely that the changes in ψ could be produced by screening alone, there is probably also some binding of divalent ions to the membrane. If we assume that ψ in 1 mM Ca^{2+} is -75 mV ($\sigma = 1$ charge per 181 \AA^2), a 20-mV fall in ψ would follow a 20% decrease in the number of excess negative charges on the membrane surface (to $\sigma = 1$ charge per 302 \AA^2).

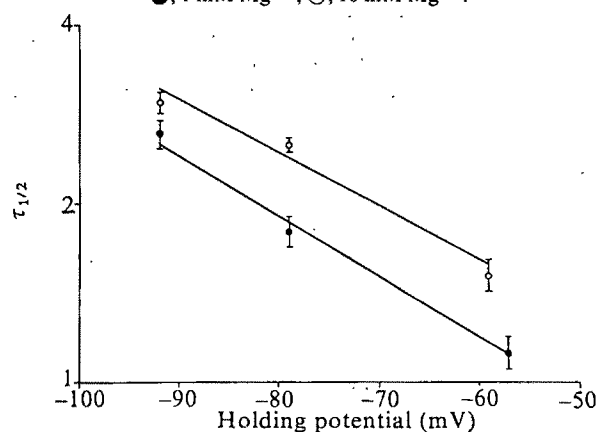
Any alteration of the surface potential will change the concentration profiles of ions between the bulk solutions on the two sides of the membrane, and thus alter the single channel conductance. In all experiments in 10 mM Mg^{2+} and Ca^{2+} we noticed a decrease in the average peak height of m.e.p.s. This result agrees with experiments showing, by noise analysis, a decrease in the single channel conductance at increased $[\text{Ca}^{2+}]$. (C. Lewis and C. F. Stevens, personal communication.)

Inspection of Figs 1 and 2, together with data not reproduced, suggests that the lines connecting the data points are not parallel, they often seem to converge or diverge at the most negative holding potentials. This could be an artefact caused by the distribution of the endplate, which extends for an appreciable fraction of a length constant along the fibre. Therefore, when the

fibre is clamped away from the resting potential, there is a voltage gradient between the current-passing electrodes and the margins of the endplate. If the resting potential falls, as is almost inevitable following repeated periods of current injection, then the voltage gradient increases, and this may lead to errors in relating the observed decay half-times to the potential set by the clamp near the centre of the endplate.

In summary, the results are consistent with the idea that the membrane at the muscle endplate has a net negative external surface charge. The lowering of the surface potential by screening and/or binding of divalent ions increases the voltage gradient within the membrane. The increased voltage gradient within the membrane slows the time course of the decline of the ACh-induced conductance change. These results have implications for other work. For example, experiments in which the Ca^{2+} and Mg^{2+} levels are manipulated to change the quantal outputs from the stimulated nerve terminals will also be changing the time course of the post-junctional current flows, unless the total concentrations of divalent cations are maintained at a constant

Fig. 2 Effects of changing membrane potential and $[\text{Mg}^{2+}]$ on the $\tau_{1/2}$ of the m.e.p.c. Solutions are as in Fig. 1b with Mg^{2+} substituted for Ca^{2+} . Bars represent \pm s.e.m. ●, 1 mM Mg^{2+} ; ○, 10 mM Mg^{2+} .



level. The post-junctional effects must be considered carefully when evaluating the results. The effects of negative surface charges near the ACh receptor will change the local concentrations of all charged substances near the surface of the membrane according to the Boltzmann distribution:

$$[i^N]_0 = [i^N] \exp[-NQ\psi/kT] \quad (2)$$

where $[i^N]$ is the concentration of I in the bulk solution $[i^N]_0$, the concentration at the membrane surface, and N is the valance. For example, if the ψ at the outer membrane surface is -70 mV, a positive univalent ion, like ACh, will be 15.4 times more concentrated at the surface. A positive divalent ion, like tubocurarine, will be concentrated more than 200-fold.

This study was supported by a grant from the NINCDS, and by an Institutional Research Support Grant from the US Public Health Service. The authors thank Mr David Attwell for discussion.

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Received 26 September; accepted 25 October 1977.

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Phospholipase A and the mechanism of action of aldosterone

ALDOSTERONE stimulates Na transport across the renal tubule and this effect is also seen in 'model' epithelia such as amphibian urinary bladder, skin and colon¹⁻³. This hormone is thought to act by inducing the formation of proteins through nuclear transcription⁴. Their nature and actions are unknown but it has been suggested that one such protein may act like a 'permease' and increase the permeability of the apical side of the epithelial cells or they could stimulate the activity of the Na transport pump. Goodman *et al.*⁵, have suggested that "some key enzyme" which increases the turnover of fatty acids may be involved and this could be phospholipase. We report here the identification of phospholipase A in toad bladder and frog skin epithelial cells and in their incubation media. Mepacrine, an inhibitor of phospholipase A, blocked the response to aldosterone on Na transport.

Toad urinary bladder and frog skin were used. Phospholipase activity was determined in tissue extracts and fluids using phosphatidyl[N-methyl]¹⁴C choline 60mCi mmol⁻¹ (Amersham-Searle) as a substrate (Table 1). Phospholipase activity was determined in tissue or cell homogenates by incubation, in 2-4 ml of Ringer solution containing 2-3 nmol of ¹⁴C-phosphatidylcholine for 2 h at 22 °C. Enzyme activity in the fluids bathing the frog skin and toad bladder preparations was determined by incubating the tissues in Ringer solution and after a specified time removing 4 ml of fluid from both sides, and incubating this with the labelled substrate. The enzyme reaction was terminated by addition of a 2:1 chloroform-methanol mixture and 1 ml of the aqueous phase was counted in a liquid scintillation counter.

Homogenates of the toad bladder and frog skin had phospholipase activity and this was localised in the separated epithelial cells (Table 1). No significant activity

was found in the liver or skeletal muscle of the toads. Gentle homogenisation of the isolated epithelial cells resulted in partial release of the enzyme in the first supernatant fraction (Table 1). The remaining activity appeared in the aqueous following sonication of the pellet. If, however, the tissues were initially sonicated, nearly all the activity could be identified in the supernatant separated by high speed centrifugation. These results suggest that while the activity may be sequestered, it is readily released, and is soluble.

We incubated bladder and skin preparations in Ringer and assayed the fluid that bathed each surface (Table 2). These solutions could also hydrolyse ¹⁴C-lecithin and the total amount of activity that was released in 3 h far exceeded that stored in the tissues. However, it is possible that endogenous unlabelled lecithin could be interfering with the tissue assays, resulting in an underestimate, though this seems unlikely in the supernatants separated by high speed ultracentrifugation. We also identified the enzyme material in the mucosal fluid bathing the toad's colon (which transports Na *in vitro*³) (Table 2), and in its urine (1 ml (14 samples) hydrolysed 90 ± 14 p mol ¹⁴C-lecithin⁻¹). Some bacteria form phospholipases so that we also included either gentamicin (Schering; 100 µg ml⁻¹) or penicillin (Pfizer, 100 U ml⁻¹) plus streptomycin (Pfizer, 100 µg ml⁻¹) in the bathing and incubation media, but they did not decrease the accumulated enzyme activity. In the bladder the rate of secretion was asymmetrical, more appearing in the mucosal than serosal fluid. In the skin a consistent pattern was not seen.

The products of the reaction of the enzyme secreted by the toad bladder epithelial cells were identified using thin

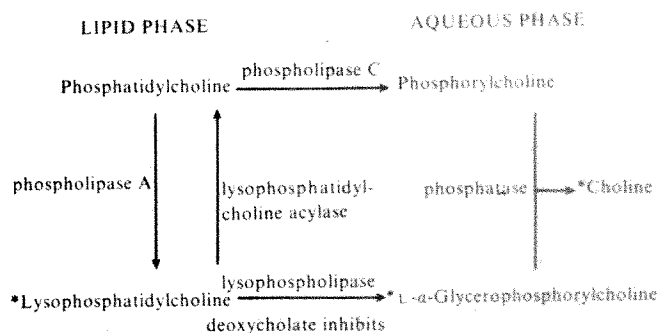


Fig. 1 The possible pathways for phosphatidylcholine metabolism and the products (*) which were identified in response to the action of the phospholipase secreted by amphibian epithelia. To identify the products of phospholipase action the lipid and aqueous phases were each subjected to thin layer chromatography. The two phases were separated and taken to dryness under reduced pressure. The remaining material was dissolved in 200 µl of methanol and 20 µl of this solution was applied to thin layer plates. Chromatographic separation of the phospholipids (lipid phase) was carried out in a solvent mixture consisting of chloroform, methanol, water (65:25:4) on silica gel G plates. The fractions were visualised by exposure to iodine vapour and identified by comparing them to appropriate reference substances. After the disappearance of the iodine staining the areas of the thin layer corresponding to phosphatidylcholine and lysophosphatidylcholine were scraped and eluted with methanol directly into vials and counted in the liquid scintillation spectrometer. The quantity of silica gel of each scraped area was constant. The relative mobilities (R_f) of these phospholipids in the solvent system used were: phosphatidylcholine, 0.34; lysophosphatidylcholine, 0.15. The labelled substrate, ¹⁴C-phosphatidylcholine, when chromatographed on thin layer plates alone was found to be >99% pure. The water soluble products were chromatographed on cellulose sheets (Kodak) using a solvent system of chloroform, methanol, ammonia (65:45:8) and visualised by spraying with a modified molybdate solution and exposing the plates to ultraviolet light for 30 min. The areas corresponding to phosphorylcholine, L-α-glycerophosphorylcholine and choline were scraped off and eluted in counting vials as described above. The R_f values were: phosphorylcholine, 0.02; L-α-glycerophosphorylcholine, 0.13 and choline, 0.40.

layer chromatography (Fig. 1). Small amounts of choline were present but the principal component in the aqueous fraction behaved chromatographically like L- α -glycerophosphorylcholine. The formation of this lipid could be prevented by the presence of 5 mM deoxycholate, which inhibits lysolecithinase⁶. Lysophosphatidylcholine (lysolecithin) accumulated in the lipid phase in the presence of deoxycholate but could not be detected in its absence. Thus the primary product of the enzymatic reaction is apparently lysophosphatidylcholine, which is formed as a result of the action of phospholipase A on phosphatidylcholine, but this is apparently normally converted to water soluble L- α -glycerophosphorylcholine. The 'lecithin-lysolecithin cycle' has previously been identified in homogenates of toad bladder⁷.

It has been suggested that a phospholipase may be involved in the action of aldosterone on epithelial membranes⁸. This could involve an increased rate of turnover of the enzyme or an increase in its activity. The demonstration of such changes may be expected to be a problem as the enzyme is readily inactivated. Thus in the bladder the secreted activity less than doubles between 3 and 18 h incubation (Table 2). Indeed, we were unable to demonstrate a change in secretion of the enzyme in response to aldosterone in the bladder. However, in another target tissue, the frog skin, accumulation increased fourfold. Frog skin moults in the presence of aldosterone, though both responses are superimposed and involve the same types of cell⁹. The morphological change may favour the release or stability of the enzyme.

Phospholipase A is inhibited by mepacrine^{9,10}; at 10^{-6} M mepacrine inhibited the *in vitro* activity of the enzyme secreted by the toad bladder by 35%. Mepacrine also completely inhibited the effects of aldosterone on Na

Table 1 Phospholipase activity in isolated epithelial cells from frog (*Rana pipiens*) skin and toad (*Bufo marinus*) urinary bladder

	¹⁴ C-phosphatidylcholine hydrolysed (pmol h ⁻¹)
Epithelial cells, toad bladder	
a, Whole homogenate, sonicated (8)	64±8
Boiled (6)	7±0.7
b, 'Mild' homogenisation and extraction (6)	
Supernatant (150,000g for 1 h)	71±8
Pellet sonicated	
Supernatant (150,000g for 1 h)	67±11
Pellet suspension	32±9
c, Cells immediately homogenised and sonicated (6)	
Supernatant (150,000g for 1 h)	138±21
Pellet resonicated	
Supernatant (150,000g for 1 h)	14±3
Pellet suspension	17±5
Isolated epithelium, frog skin	
Whole homogenate, sonicated (6)	60±9
Boiled (5)	11±1.6

The toads weighed about 200 g. The bladders (two hemi-bladders, about 200 mg whole tissue) were perfused with Ringer to remove the blood. The bladder epithelial cells were separated by incubating each hemi-bladder sac preparation in Ringer (in mM, NaCl, 111; KCl, 3.35; CaCl₂, 2.7; NaHCO₃, 4.0 and glucose 5.0) in the absence of added Ca²⁺ and containing 2 mM EGTA, for 75 min. The cells were separated by gently massaging the sac, pooled and centrifuged for 15 min at 2,500g. Pieces of frog skin epithelium were separated by exposing the inner surface to collagenase (Sigma, 250 µg ml⁻¹) under about 50 cm hydrostatic pressure for about 2 h. The isolated bladder epithelial cells were resuspended in 2 ml of a solution containing 4 mM NaHCO₃ and 5 mM CaCl₂ (pH about 8.0), and incubated with 2–3 nmol of phosphatidyl[N³-methyl-¹⁴C]choline for 2 h at 22 °C. The solution was then extracted with 19 ml of 2:1 chloroform-methanol and 1 ml of the aqueous was counted in a scintillation counter. Assays were corrected for a reaction 'blank' which was small and amounted to about 0.4% of the total phosphatidylcholine present. Frog skin epithelium (20 cm², 424 mg whole skin) was assayed similarly. Results are means ± s.e. Numbers of experiments carried out shown in parentheses.

Table 2 Secretion of phospholipase by frog skin and toad bladder

	¹⁴ C-phosphatidylcholine hydrolysed (pmol h ⁻¹)
Toad bladder: Mucosal side 3 h (8)	113±20
Serosal side 3 h (6)	46±11
Mucosal side 3 h, boiled (6)	5±1
Saline treated toads:	
Total (mucosal and serosal) 3 h (8)	58±18
Frog skin: Epidermal side 3 h (10)	64±10
Corium side 3 h (10)	67±17
Toad colon: mucosal side 3 h (5)	39±6
Effect of 10 ⁻⁶ M aldosterone; (total secretion)	
Toad bladder; 18 h incubation*	
Control (6)	260±55
Aldosterone (6)	274±60
Frog skin; 18 h incubation	
Control (10)	318±25
Aldosterone (10)	1,267±198†

Toad hemi-bladders (and colons) were prepared as sacs. The short-circuit current (µA per 100 mg) for the 18 h incubation was 158±20 for the control, and 555±62 for aldosterone. The frog skins were either tied onto lucite cylinders (surface area 10 cm² = 200 mg) or for testing the effects of aldosterone, prepared in Ussing-type chambers (3 cm² = 60 mg). The short-circuit current after 18 h was (µA cm⁻²) 17±2 in the control and 42±8 in the aldosterone treated skin. Values are mean ± s.e., pmol hydrolysed in fluids bathing 100 mg tissue. Number of experiments shown in parentheses.

*We were also unable to see a stimulation of enzyme activity by aldosterone even for the shorter time period of 3 h either initially or following an 18 h preincubation.

†P < 0.01 for difference from control.

transport in the toad bladder (Table 3) though it had no significant effect on the natriuretic response to vasopressin (ADH). Phospholipase A₂ is the rate limiting step in the formation of prostaglandins. This enzyme releases free

Table 3 Effects of inhibitors of phospholipase A and prostaglandin synthetase on the response of the toad bladder (*in vitro*) to aldosterone (10⁻⁷ M) and vasopressin (AVP, 10 mU ml⁻¹)

		Short circuit current (µA per 100 mg wet wt)		
		Initial	Final 5 h	Mean difference
I	a, Control (6)	276	251	-25±24
	+10 ⁻⁶ M Mepacrine	229	219	-10±28
	b, Aldosterone (6)	345	762	434±118*
	+10 ⁻⁶ M Mepacrine	357	452	96±49
	c, Aldosterone (6)	318	764	446±127*
	+5 × 10 ⁻⁶ M Mepacrine	424	334	-90±46
	II a, Control (12)	168	198	30±16
	+10 ⁻⁵ M Indomethacin	207	98	-109±22†
	b, Aldosterone (6)	295	775	480±120*
	+10 ⁻⁵ M Indomethacin	350	439	89±46
III	a, Control (12)	234	252	18±30
	+10 ⁻⁵ M Mefenamic acid	244	159	-85±42
	b, Aldosterone (6)	231	681	450±111†
	+10 ⁻⁵ M Mefenamic acid	291	437	146±62
	IV a, AVP (6)	251	621	370±34†
	+10 ⁻⁶ M Mepacrine	219	582	363±18†
	b, AVP (12)	198	715	517±106†
	+10 ⁻⁵ M Indomethacin	98	548	450±74†
	c, AVP (12)	252	820	568±119†
	+10 ⁻⁵ M Mefenamic acid	159	588	429±69†

Toad hemi-bladders were prepared as sacs. Paired bladder lobes were used. The number of paired experiments are in parentheses. Short circuit current reflects Na transport. In IV the tissues were incubated for 5 h in the presence or absence of the drug, before adding the AVP.

*P < 0.02 for differences from initial period.

†P < 0.01.

fatty acids which are substrates for prostaglandin synthesis. It was therefore also interesting to observe that mefenamic acid and indomethacin which inhibit prostaglandin synthetase¹¹ also inhibited the action of aldosterone on Na transport, though the response to vasopressin was unaffected (Table 3).

The effect of aldosterone on transepithelial Na transport thus seems to be related to the actions of two enzymes, phospholipase A and prostaglandin synthetase. Both of these are known to be present in this tissue and are known to be involved in the common chain of events that mediates (this paper and ref. 12) prostaglandin synthesis. The mechanism of aldosterone action is unclear, but it could be inducing the formation, activation, or release of phospholipase A. It has recently been shown that RNA and protein synthesis are necessary for expression of phospholipase action in mouse fibroblasts¹³. We do not know by which process prostaglandins could be influencing the response but some are known to increase active Na transport across these membranes^{14,15}.

This work was supported by the US NSF. Dr L. A. Barker kindly helped and advised us in the identification of phospholipase A.

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Received 8 July; accepted 11 November 1977.

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Inactivation of interferon mRNA in the shutoff of human interferon synthesis

INDUCTION of interferon synthesis in cultured cells by $rI_n \cdot rC_n$ has been intensely studied because of its attractiveness as a model for gene expression in mammalian cells and its possible significance to medicine. Exposure of human fibroblast FS-4 cells to $rI_n \cdot rC_n$ results in interferon (IF) synthesis which persists for 6 h and then abruptly shuts off^{1,2}. The rapidity of this shutoff indicates that the cessation of IF mRNA transcription is unlikely to be the primary control. Moreover, studies with metabolic inhibitors³⁻⁷ have suggested that a post-transcriptional event is involved. To evaluate the role of post-transcriptional control in the shutoff of IF production, we measured directly the level of IF mRNA activity in a strain of human fibroblast HF 926 cells during the IF induction process. This was achieved by the development of a highly efficient and reproducible heterologous whole cell translational system. We report here that, using this system, we have found a significant decrease in the IF mRNA activity during the shutoff of IF protein synthesis.

The translational system consists of the direct application of isolated human RNA to Syrian hamster embryo (SHE) cells. Absolute species specificity between the human and hamster interferons allows for the bioassay of human IF

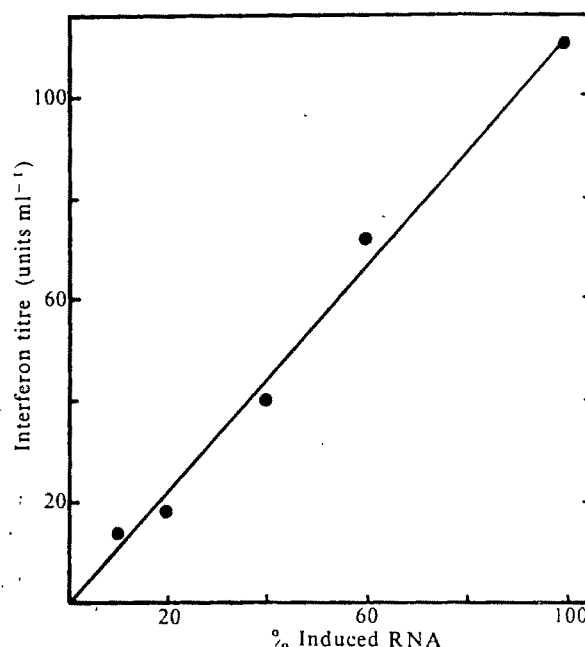
Table 1 Effect of actinomycin-D pre-incubation and RNA facilitators on interferon mRNA Translational efficiency

Treatment	Incubation time (h)	Facilitator	IF titre (units ml ⁻¹)	% Efficiency
None	-	CaCl ₂	13	6.25
Actinomycin D	5	CaCl ₂	22	11.0
Actinomycin D	5	DEAE-Dextran	4	2.0
Actinomycin D	10	CaCl ₂	22	11.0
Actinomycin D	15	CaCl ₂	41	20.0

Confluent monolayers of Syrian hamster embryo cells (SHE) in 75 cm² flasks with $3-4 \times 10^6$ cells per flask were treated with 0.065 μ g ml⁻¹ actinomycin D in maintenance media for the times indicated. After actinomycin treatment, 50 μ g ml⁻¹ cytoplasmic RNA, obtained from $rI_n \cdot rC_n$ -induced HF-926 cells by a modified Penman procedure¹³, were applied to the SHE cells with either 50 μ g ml⁻¹ of DEAE-dextran or 30 mM CaCl₂ in 5 ml of HEPES-phosphate-buffered saline (PBS) buffer, pH 7.0. After incubating at 37 °C for 1 h, the HEPES-PBS buffer was removed from all flasks and replaced with 10 ml of maintenance media. Translation was allowed to proceed for 5 h and the media was collected for assay.

that has been translated in the heterologous SHE cells. Translation is dependent on application of the RNA with a substance which facilitates the RNA uptake. Both DEAE-dextran⁸ and CaCl₂ (refs 9, 10) have been used successfully to enhance the infectivity of viral nucleic acids. We adapted these procedures for mRNA. CaCl₂ was a more effective facilitator than DEAE-dextran in both translational efficiency and reproducibility (Table 1). Pretreatment of the recipient SHE cells with actinomycin-D before addition of human IF mRNA was not essential, but this treatment increased translational efficiency, particularly with long preincubation times. Presumably, this enhancement is the consequence of endogenous mRNA degradation in the SHE cells with the resultant increase in translational capacity. Efficiency of translation can be estimated by comparing the amount of human IF translated by the SHE cells from a given quantity of added human RNA to the amount of IF

Fig. 1 Cytoplasmic RNA extracted by a modified Penman procedure¹³ from HF 926 cells 3 h after induction with $rI_n \cdot rC_n$ was mixed with cytoplasmic RNA from mock induced HF 926 cells at the shown ratios. The total concentration of RNA in each sample was 50 μ g ml⁻¹. All points represent the average of duplicate assays.



produced from an equivalent number of human cells from which the RNA was isolated (Table 1). For instance, in a typical experiment, one 1/2 gallon roller bottle (690 cm^2 , $2\text{--}3 \times 10^7$ cells) of induced human cells produced approximately 10,000 IF units, whereas approximately 600 IF units (6% efficiency) were produced by the SHE cells on translation of the mRNA isolated from this roller bottle. Depleting the SHE cells of Ca^{2+} before addition of human RNA also increases IF mRNA translation (Table 2). Interferon activity was always associated with the poly A mRNA fraction retained on oligo dT-cellulose and not with the flow-through fraction. No IF production was found when RNA from uninduced human cells was used in translation.

When cytoplasmic RNA extracted from $\text{rI}_n\text{-rC}_n$ -induced HF 926 cells was applied to Ca^{2+} -depleted SHE cells using the CaCl_2 precipitation technique, the IF translation was found to be independent of the absolute RNA concentration. In the concentration range of $10\text{ }\mu\text{g ml}^{-1}$ to $100\text{ }\mu\text{g ml}^{-1}$, the IF yield remained constant. But, when RNA from induced cells was diluted with varying amounts of carrier RNA while keeping the total RNA concentration constant at $50\text{ }\mu\text{g ml}^{-1}$, IF production varied linearly with the proportion of IF mRNA in the applied RNA sample (Fig. 1). These observations can be explained on the basis of a limitation in the RNA uptake of SHE cells. If the uptake capacity is limited, then the absolute amount of mRNA translated by the SHE cells is fixed when the amount of applied RNA exceeds this capacity. Therefore, in saturating conditions (over $10\text{ }\mu\text{g RNA per ml}$) the SHE cell translational system responds proportionately not to absolute dosage of IF mRNA but rather to its relative concentration in the preparation (equivalent to 'specific biological activity' of IF mRNA).

Kinetics of the IF protein synthesis measured in HF 926 cells after 1 h induction with 0.4 mM ($130\text{ }\mu\text{g ml}^{-1}$) $\text{rI}_n\text{-rC}_n$ in physiological salt buffer (0.15 M NaCl , 0.01 M PO_4 , 0.001 M MgCl pH 7.2), is shown in Fig. 2a. The rate of IF synthesis reached a maximum at about 3–4 h after induction and was largely shut off by 8 h. In many of these experiments, especially when a lower concentration of $\text{rI}_n\text{-rC}_n$ was used for induction, there was no detectable synthesis at 8 h. These findings corroborate those of Vilček and his colleagues¹¹ for FS-4 cells.

Total cytoplasmic RNA was extracted according to a modified Penman procedure^{12,13} from the same cells and at the same time points as in Fig. 2a. After deproteination, each RNA sample was adjusted to a concentration of $50\text{ }\mu\text{g ml}^{-1}$, 30 mM CaCl_2 and translated in Ca^{2+} -depleted SHE cells. The kinetics of IF mRNA activity are given in Fig. 2b. Similar results were obtained when DEAE-dextran was used to facilitate RNA uptake. The appearance of IF mRNA in the human cells after exposure to $\text{rI}_n\text{-rC}_n$ is very rapid, peaking at 2–3 h post-induction. More significantly, there is an abrupt decline in IF mRNA activity which corresponds to the shutoff of the IF synthesis.

Sehgal *et al.* have shown that interferon shutoff can be prevented when an RNA inhibitor such as actinomycin D or 5,6-dichloro-1- β -D-ribofuranosylbenzimidazole is given to the cells after induction⁴. In these conditions, synthesis of IF can

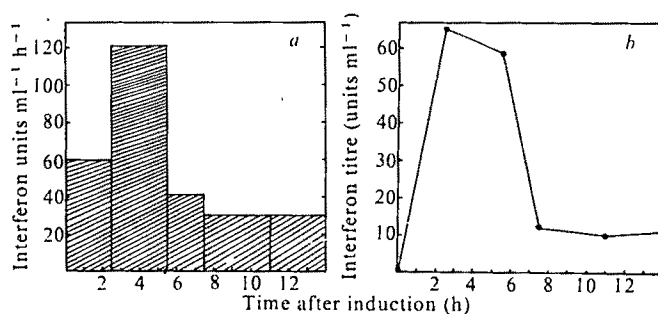


Fig. 2 HF 926 cells in 1/2 gallon roller bottles were induced with $0.4\text{ mM rI}_n\text{-rC}_n$ for 1 h then re-fed with D-MEM with 2% foetal calf serum. Media was collected for measuring IF titres and the cells extracted for RNA at 2½, 5½, 7½, 11, and 14 h. Similar results were obtained in two additional experiments. a, Rates of IF synthesis following induction. b, IF mRNA activity following induction.

persist beyond 20 h indicating that the human IF mRNA is a stable species with a half life on the order of 15 h. In view of their finding, it is unlikely that the rapid decline in IF mRNA activity is the result of a natural decay process but is more likely to be the result of a specific inactivation event.

IF mRNA activities during the induction of IF have been examined in two studies by whole cell translational systems. Kronenberg and Friedman¹⁴ measured $\text{rI}_n\text{-rC}_n$ induced L cell IF mRNA by translation in Vero cells. But, they did not attempt explicitly to correlate the IF mRNA activities with IF protein levels nor did they attempt to study the shutoff process. At 3 h after induction, when no IF was produced, they detected high levels of IF mRNA whereas at 16 h, when IF was synthesised at maximal rate, they observed a reduction in the levels of IF mRNA. These observations indicate that the system of IF induction in mouse cells is different than in human cells and, therefore, complicate comparisons between these two cell types. Furthermore, since no IF mRNA determinations were made during the shutoff of mouse IF, no conclusions can be made concerning the relationship between IF shutoff and IF mRNA activities.

Reynolds and Pitha¹⁵ studied both human IF production and IF mRNA activities using the avian cell translational system originally developed by DeMayer-Guignard *et al.*¹⁶. In contrast to our findings they did not observe a decrease either in the rate of IF production or in the levels of IF mRNA. More recently, Raj and Pitha¹⁷ observed that IF production in human fibroblasts declines 90% by 6–9 h after induction.

An important consideration in the interpretation of these data is the validity of the translational system. An ideal translational system should translate exogenous mRNA with fidelity and high efficiency, thereby reflecting the functional state of the IF mRNA in the originally induced cell. Translation of the human mRNA by another mammalian cell, such as the hamster, should offer the best prospects in this respect.

Work is in progress to identify the regulatory elements involved in the inactivation of IF-mRNA. Such a repression mechanism may be of broader biological relevance.

These results were reported at the 77th annual meeting of the American Society for Microbiology¹⁸. We thank Mr Stanley Lin for many helpful discussions. This research was supported in part by US NIH (grant GM 166066).

Note added in proof: Sehgal *et al.* and Cavaliere *et al.* using the *Xenopus laevis* oocyte translational system have also observed^{19,20} the rapid decline in IF mRNA activity during the shutoff of human IF synthesis.

Table 2 Effect of Ca^{2+} -depletion on the translation of human interferon mRNA in SHE cells

Treatment	IF titre (units ml^{-1})
Control (media only)	20
Dulbecco's PBS-HEPES (30 min)	19
Ca^{2+} -free PBS-HEPES (30 min)	76

Translation was as for Table 1 except that before addition of the RNA, the SHE cells were either untreated or incubated with Ca^{2+} -free or normal PBS-HEPES buffer for 30 min.

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Received 22 July; accepted 24 October 1977.

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Bleomycin causes release of nucleosomes from chromatin and chromosomes

THE antibiotic bleomycin is effective against certain human neoplastic diseases¹. Interaction of purified DNA with bleomycin results in the release of free bases from the DNA backbone^{2,3}, disruption of phosphodiester linkage, and destruction of the deoxyribose moiety⁴. Bleomycin-induced DNA strand scissions, both single- and double-strand, were found in bacterial as well as in mammalian systems^{5,6}. But, all previous work on bleomycin has dealt with DNA molecules extracted from treated cells, and analysed on sucrose density gradients. This is not sensitive enough to probe the action of bleomycin with respect to chromatin structure. It is now known that chromatin is composed of a number of repeating subunits connected by a continuing DNA duplex analogous to 'beads on a string'⁷. The bead-like structure, termed nucleosome or nu-body, contains 140 base pairs and eight histone molecules^{8,9}. The DNA between nucleosomes, about 40-60 base pairs in length, is referred to as the linker DNA. Both DNAs in the nucleosomes and in the linker are susceptible to nuclease digestion, but the latter is more sensitive¹⁰⁻¹². We have analysed the effects of bleomycin on chromatin with special reference to the characterisation of the breakage sites in a number of mammalian cell systems. The results reported here indicate that bleomycin is one of the few non-enzymatic chemicals which can be used to study the chromatin structure, and that the action of bleomycin is not identical to that of nucleases—bleomycin does not digest nucleosome DNA but attacks only the linker DNA.

When nuclei isolated from cultured Chinese hamster cells (line CHO) were incubated with various concentrations of bleomycin, followed by deproteinisation and gel electrophoresis on agarose, a characteristic series of DNA bands was obtained (Fig. 1). DNA isolated from untreated nuclei showed a high molecular weight (gel 3), indicating that endogenous nucleases had no significant effect on native DNA. The DNA isolated from the nuclei treated with higher concentrations of bleomycin contained more DNA fragments with lower molecular weight than that from

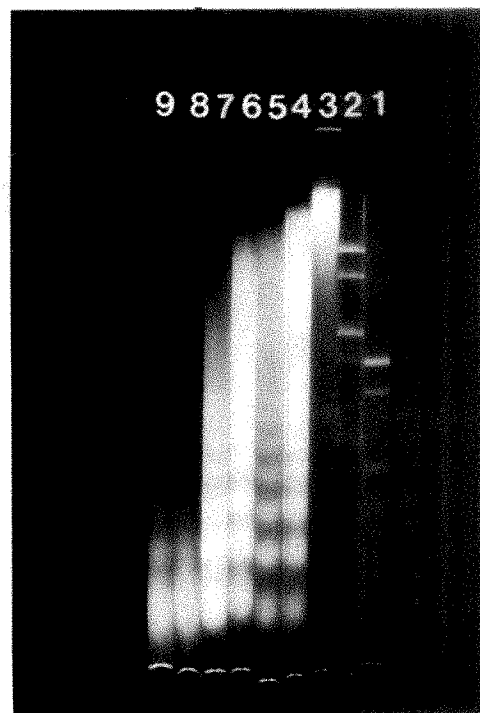


Fig. 1 Agarose gel analysis of DNA digested *in situ* with bleomycin. Approximately 10^7 CHO nuclei isolated using the method described previously²⁰ were incubated in 400 μ l of reaction mixture containing 15 mM Tris-HCl, pH 8.0, 0.34 M sucrose, 0.5 mM EDTA and 15 mM 2-mercaptoethanol and various concentrations of bleomycin at 22 °C for 2 h. The reaction was terminated by the addition of an equal volume of solution containing 0.15 M NaCl, 15 mM EDTA, 50 mM Tris-HCl, pH 8.25, 0.3% sarcosyl NL 97. The mixture was treated with 100 μ g ml⁻¹ of heated (100 °C for 5 min) RNase A at 37 °C for 1 h. DNA was extracted by deproteinisation with phenol saturated with 0.15 M NaCl, 0.1 M EDTA followed by chloroform:isoamylalcohol (24:1,v/v), and precipitated with 2.5 volumes 100% ethanol. Agarose (1.4%) was made in E buffer containing 40 mM Tris-HCl, pH 8.0, 5 mM Na-acetate, 1 mM EDTA. Electrophoresis was run in E buffer at 4 mA per gel. Gel (1) *Hae*III-digested PM 2 DNA, (2) *Hind*III-digested PM 2 DNA, (3-9) CHO DNA; (3) control, no bleomycin added, (4) 1.5 μ g/ml, (5) 15 μ g ml⁻¹, (6) 300 μ g ml⁻¹, (7) 1 mg ml⁻¹, (8) 2 mg ml⁻¹, and (9) 3 mg ml⁻¹ bleomycin.

nuclei treated with lower concentrations of bleomycin, indicating that the fragmentation of nuclear DNA is dose dependent (gels 4-9).

The molecular weight of each band (Fig. 1) was determined by three methods. (1) Construction of a calibration curve by plotting the migration distance of the PM 2 phage DNA fragments digested by restriction enzymes *Hind*III and *Hae*III (Fig. 1, gel 1, 2) against their molecular weights (data not shown). The lengths of the second band (from the bottom of the gels) to the 5th band, averaged from three independent experiments, were 343 ± 7 , 535 ± 15 , 693 ± 15 , and 890 ± 10 base pairs, respectively. These values correspond well to a monomer unit length of 175 ± 4 base pairs. (2) Linear regression analysis of the logarithms of the molecular weight of the restriction fragments plotted against mobilities. The repeated unit length was found to be 170 ± 8 base pairs. (3) Subtracting the length of each band from that of the next higher band. Using this method, the unit length was found to be 182 ± 10 base pairs. The average of these three determinations yielded a unit length of 175 ± 8 base pairs, which is in good agreement with the nucleosome unit length reported by other investigators^{13,14} using nuclease digestions. Bleomycin, therefore, acts on chromatin in a manner analogous to that of nucleases.

Further analyses showed, however, that the modes of action on chromatin DNA between nucleases and bleomycin are not exactly the same. Nucleases have been shown

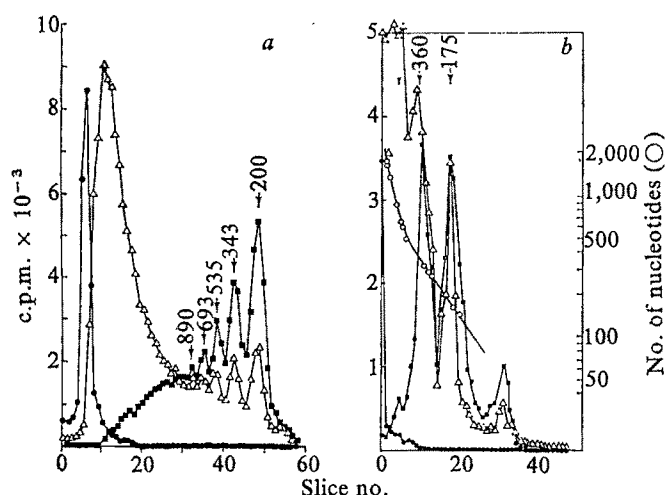


Fig. 2 Gel electrophoretic patterns of DNA isolated from bleomycin-treated CHO nuclei. CHO cells were labelled with ^3H -TdR ($0.5 \mu\text{Ci ml}^{-1}$, 22 Ci mmol^{-1}) for 24 h. Procedures for isolation of nuclei and digestion by bleomycin are described in Fig. 1. ●, Control, no bleomycin; △, $1.5 \mu\text{g ml}^{-1}$ bleomycin for 5 h; ■, $300 \mu\text{g ml}^{-1}$ for 5 h. a, DNA samples were electrophoresed in non-denaturing conditions as described in Fig. 1. b, Corresponding samples as run in denaturing conditions, that is, 5% acrylamide in 99% formamide²¹. The marker DNAs (○) shown in (b) are *Hind*III- and *Hae*III-digested PM 2 DNA fragments^{22,23}. The arrows represent the corresponding numbers of base pairs (a) or nucleotides (b) as calibrated from the same DNA markers co-electrophoresed.

to digest intra-nucleosomal DNA and produce a series of bands with a multiplicity of 10 nucleotides as analysed by a denaturing gel¹⁵⁻¹⁷. We did not obtain similar results when bleomycin was used.

Figure 2 shows gel electrophoretic patterns of radioactively-labelled DNA isolated from bleomycin-treated nuclei or control nuclei. The specific activity of DNA was approximately $20,000 \text{ c.p.m. } \mu\text{g}^{-1}$, sensitive enough to detect an amount as small as 5 ng. The control sample gave no detectable amount of DNA with molecular weight less than 5,000 base pairs. The treated sample showed DNA in a series of bands with multiplicity of 175 base pairs as described previously.

When the same samples shown in Fig. 2a were denatured and separated on denaturing gels, a characteristic pattern emerged (Fig. 2b). In the bleomycin-treated samples, only bands with sizes of 50, 175 and 360 nucleotides were present. The minor band (50 nucleotides in length as analysed by extrapolation of the calibration curve) was not present in the undenaturing DNA gel. This fragment must, therefore, have come from a higher molecular weight, double-stranded duplex containing single-stranded nicks.

That the double-stranded, high molecular weight DNA contains single-strand nicks can also be seen by comparing the same sample ($300 \mu\text{g ml}^{-1}$, 5 h) electrophoresed in non-denaturing (Fig. 2a) and denaturing conditions (Fig. 2b). Figure 2a shows that about 50% of the counts migrated with molecular weights higher than 500 base pairs. But, when this sample was denatured and separated in denaturing gel, the high molecular weight DNA (>500 base pairs) disappeared and was replaced by monomeric and dimeric fragments. From these results, we conclude that the high molecular weight DNA duplex contained single-strand breaks spaced regularly, probably every 50, 175 or 360 nucleotides apart.

We detected no multiplicity of 10 nucleotides on the gels. This indicates that the nuclease-sensitive sites within nucleosomes are not sensitive to bleomycin. An explanation for this phenomenon is that bleomycin is more specific

in its recognition of sequence or structure of the DNA helix than are nucleases. Previously, we have reported that DNA with molecular weight less than approximately 12 nucleotides is resistant to bleomycin degradation, regardless of the sequence¹⁸. If DNA within the nucleosomes is kinked every 10 base pairs as proposed by Crick and Klug¹⁹, or embedded within protein molecules, then the intranucleosomal DNA may become resistant to bleomycin activity, so that no internal nicks can be created. Our interpretation is that bleomycin induces damage only in the internucleosomal linker DNA, and that the 50 nucleotide pieces probably came from nicks in the linker DNA at or near the adjacent nucleosomes. We also found that several mammalian cells in addition to CHO responded to bleomycin in a similar manner, and that the antineoplastic protein, neocarzinostatin, also yielded DNA degradation products similar to those of bleomycin. We believe that these drugs will be very useful tools in the study of chromatin structure.

This work was supported in part by the National Institute of Environmental Health Science (grant ES-01304) and the US NSF (ENV-76-82241). We thank Drs Roger Hewitt, R. T. Kovacic and James C. Wang for supplying PM 2 bacteriophage DNA.

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Received 6 September; accepted 24 October 1974.

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Dissociation of 'hybrid' isozymes on electrophoresis

HETEROMERIC or 'hybrid' isozymes can be readily demonstrated by electrophoretic techniques. They can be observed, for example, in individuals who are heterozygous for alternative alleles at an autosomal locus determining a multimeric enzyme or where multiple loci are involved in the determination of a multimeric enzyme. Heteromeric forms can also be generated by techniques such as somatic cell fusion, and by dissociation and recombination *in vitro*. The number of heteromeric isozymes displayed by a particular enzyme is related to its subunit composition. For example, in a heterozygote a dimeric enzyme exhibits one heteromeric isozyme and two homomeric isozymes, whereas a

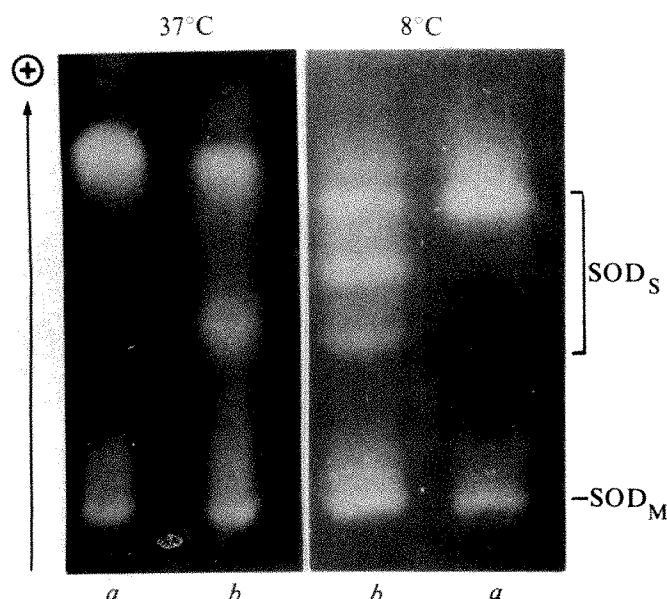


Fig. 1 Starch gel electrophoresis of SOD at 8 °C and at 37 °C using a Tris, EDTA, maleate, MgCl₂ buffer system at pH 7.4. Electrophoresis was carried out for 17 h at 5 V cm⁻¹ at 8 °C and 3.5 V cm⁻¹ at 37 °C. Temperature was regulated during electrophoresis by circulating water at the appropriate temperature through electrically insulated metal plates which were in close contact with the gel surface. The gels were stained using MTT 0.1 mg ml⁻¹ and phenazine methosulphate 0.05 mg ml⁻¹ in Tris/Cl buffer, pH 8.0 applied as an agar overlay. The samples are liver extracts from (a) an individual of phenotype SOD_s 1 and (b) an individual of phenotype SOD_s 2-1. The most cathodal zone of activity is SOD_M which does not show the same genetic variation.

tetrameric enzyme displays three heteromeric isozymes in addition to the two homomeric forms. Thus, the number of heteromeric isozymes provides a valuable method of assessing the number of subunits making up active enzyme molecules and indeed this approach has recently been used in a survey of the subunit structures of 66 different human enzymes¹. It was found that heteromeric isozymes usually occur as described here, but there are occasional exceptions where the expected 'hybrid' isozymes are unusually weak or absent. We have investigated one component of the electrophoretic separation system—that is, temperature—and its influence on the occurrence of heteromeric isozymes using soluble superoxide dismutase (SOD_s) as a test case. This was prompted by a report that this dimeric enzyme is

readily dissociated at high temperatures²; also, the heteromeric isozymes of SOD_s can be conveniently studied by electrophoretic methods. Our results suggest that temperature has a crucial effect on the occurrence of the heteromeric isozymes of this enzyme.

SOD, which catalyses the disproportionation of superoxide radicals, exists in two forms in eukaryotes³. One form, SOD_s, found in the cytosol, is a dimeric copper-zinc protein with a molecular weight of 32,000. The mitochondrial form, SOD_M, is a tetrameric manganese protein with a molecular weight of about 72,000. An electrophoretically slow genetic variant of the soluble enzyme, designated SOD_s 2-1 and attributable to heterozygosity for a variant allele SOD_s¹ (ref. 2), and the common allele SOD_s¹, has been identified⁴. In heterozygotes, three isozymes are observed under standard electrophoretic conditions. The two outer zones representing the homomeric forms, and the middle zone the heteromeric ('hybrid') form containing polypeptides determined by each allele.

A liver extract from an individual of the SOD_s 2-1 phenotype was subjected to electrophoresis in starch gels at various temperatures ranging from 8–55 °C. At 8 °C, the usual three-banded pattern was observed. At 20–30 °C, a three-banded pattern was also observed; but, whereas the homomeric isozymes were of usual activity, the heteromeric form was much less active. At temperatures above 35 °C, two bands were observed with mobilities corresponding to the two homomeric forms and displaying normal activity. The heteromeric isozyme was not present (Fig. 1). Similar results were obtained with man-mouse hybrid material derived from somatic cell fusion and from *in vitro* dissociation and recombination (Fig. 2).

A simple explanation for these findings would be that heteromeric isozymes of SOD_s are more heat sensitive than homomeric forms. Heating the SOD_s isozymes (40 °C, 17 h) in the gels using the technique of McAlpine *et al.*⁵, subsequent to their separation by electrophoresis at 8 °C, did not, however, lead to any noticeable loss of activity. Furthermore, heating a crude tissue extract at 70 °C for up to 3 h, followed by electrophoresis at 8 °C, revealed that the homomeric and heteromeric isozymes lose activity at the same rate. Thus, it seems that the disappearance of the heteromeric isozymes during electrophoresis is not due to heat lability.

A more likely explanation (similar to that proposed to account for the disappearance on electrophoresis of the asymmetric 'hybrid' forms of haemoglobin^{6,7}) is that the dimeric SOD_s isozymes are in rapid equilibrium with their constituent polypeptides at elevated temperatures:

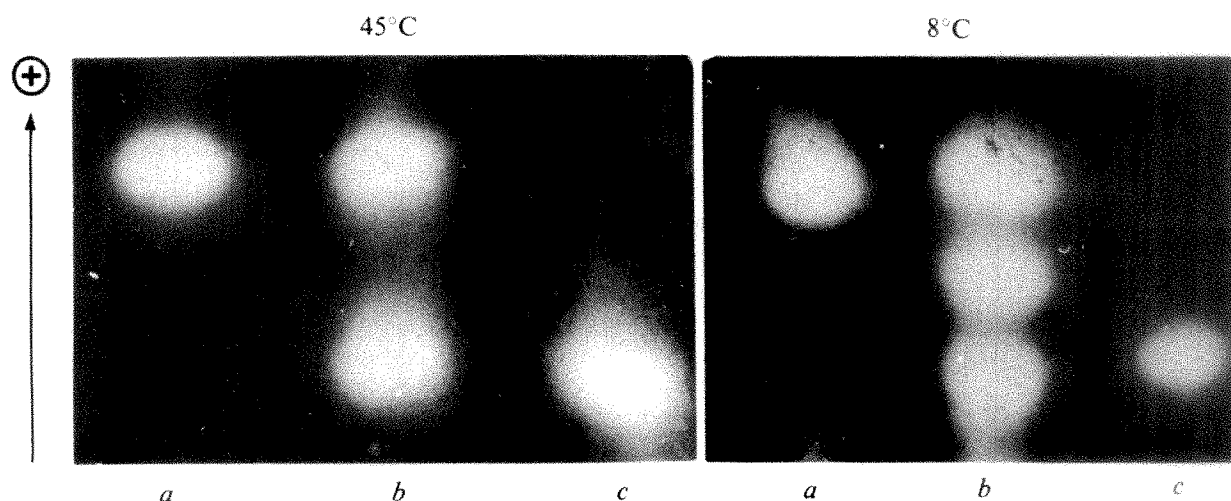
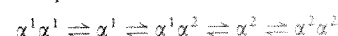


Fig. 2 Electrophoresis of SOD_s at 8 °C (5 V cm⁻¹) and at 45 °C (3 V cm⁻¹) using the procedure detailed in Fig. 1. Samples: a, human liver; b, a 'hybrid' preparation of man and mouse SOD_s prepared by *in vitro* dissociation and recombination; c, mouse liver.

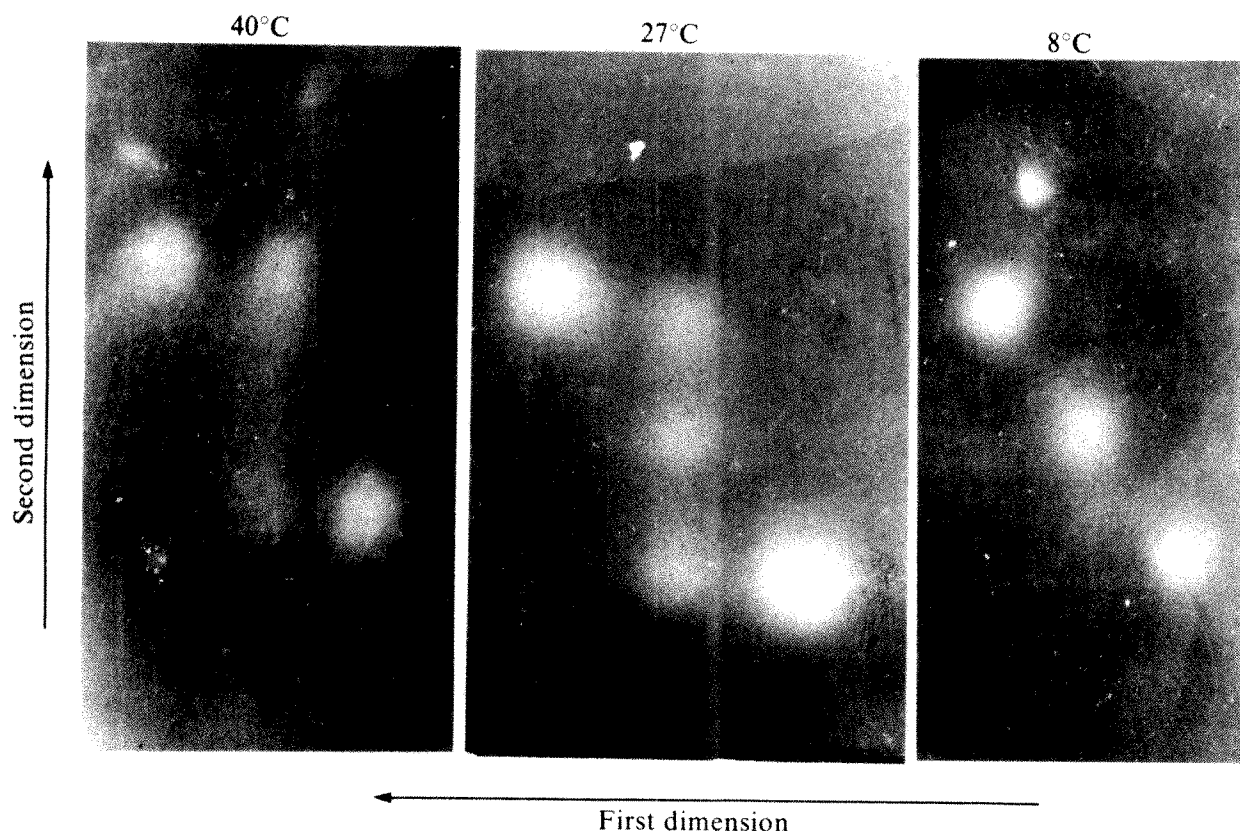
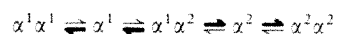


Fig. 3 Two-dimensional electrophoresis of man-mouse 'hybrid' SOD₅ isozymes. In each case electrophoresis in the first dimension was at 8 °C. In the second phase electrophoresis was carried out at 8, 27 and 40 °C. Conditions for electrophoresis and SOD detection as described in Fig. 1.

On electrophoresis, this equilibrium is disturbed due to the separation of the unlike forms, so that there is a progressive depletion of the heteromeric ('hybrid') isozyme:



In ordinary temperature conditions, the dissociation-reassociation process is presumably much less rapid, and the heteromeric SOD₅ isozyme persists.

This interpretation has been investigated using a two-dimensional technique. In the first dimension, separation was carried out at 8 °C (that is, the usual condition for SOD₅ electrophoresis) and then in the second dimension at 8 °C, 45 °C or some intermediate temperature. In one series of experiments, samples containing three isozymes of SOD₅ were separated by electrophoresis at 8 °C and then the pieces of gel containing the middle band (the heterodimeric isozyme) were cut out and subjected to electrophoresis at 45 °C. In each case—regardless of whether the heterodimer was derived from a SOD₅ 2-1 heterozygote, a somatic cell hybrid or from *in vitro* dissociation and recombination of man and mouse SOD₅—this single band was found to generate two isozymes of equal intensity and with electrophoretic mobilities identical to the corresponding homodimers. In another series of experiments, the second phase of electrophoresis was carried out using all three isozymes (the two homodimers and the intermediate heterodimer). With increasing temperature in the second phase of electrophoresis, there was a coincident apparent increase in the rate at which the heteromeric isozyme generated the homomeric bands (Fig. 3). Thus, it seems that temperature has a crucial influence on the appearance of the heteromeric isozymes of SOD₅ after electrophoresis.

We have made preliminary investigations into the effects of temperature on a limited number of other enzymes which exhibit heteromeric isozymes, but so far no example of dissociation during electrophoresis which is as clear-cut as that described for SOD₅, has been seen. A wide range of enzymes, however, remains

to be investigated as well as many other factors, such as pH, buffer ion concentration and coenzyme binding, which might affect the dissociation of isozymes during electrophoresis. Also, the condition of the sample may influence the appearance of heteromeric forms, since it has recently been shown that, although an asymmetric 'hybrid' haemoglobin band is not usually seen—for example, in A-S and A-C heterozygotes—on conventional electrophoresis, this type of band is observed when the haemoglobins are reduced and maintained in anaerobic conditions during electrophoresis⁸.

It seems particularly worthwhile to investigate any multimeric enzymes (or other proteins) which are found to show exceptional isozyme patterns in respect of 'hybrid' forms in case the hybrids are being lost by dissociation during the separation process. For instance, in the case of galactokinase there is evidence for a dimeric structure⁹, but a heteromeric isozyme with a mobility intermediate between the human and mouse isozymes has not been observed in hybrid cell lines¹⁰. Another example is enolase, a dimeric enzyme determined at three separate loci, coding for the polypeptides α , β and γ . The heterodimers $\alpha\beta$ and $\gamma\beta$ are observed after electrophoresis together with the homodimers $\alpha\alpha$, $\beta\beta$ and $\gamma\gamma$, but the expected heterodimer $\alpha\gamma$ has not been detected in either human or rat tissues¹¹.

In every unusual case, however, it will be necessary to bear in mind other reasons for the absence of heteromeric isozymes after electrophoresis. In the case of enzymes determined by X-linked loci, where only one X chromosome is functioning in each cell, there is no opportunity for unlike polypeptides to come together to form hybrid isozymes. This is illustrated by the glucose-6-phosphate dehydrogenase isozymes in females of the AB phenotype: hybrid isozymes have been observed in oocytes from such females, in which both X chromosomes are active, but not in other tissues¹². Differences in gene expression from one cell type to another also results in the absence of heteromeric forms. For example, lactate dehydrogenase (LDH) AC and BC hybrids are not found in testicular tissue. This is because only the LDH_C locus

is active in primary spermatocytes, whereas the LDH_A and LDH_B loci are expressed in other cells of the testis¹³.

Sometimes, there may be stereochemical reasons why dissimilar polypeptides do not form a hybrid. A possible example is the rare genetic variant of human LDH¹⁴. The multimeric enzymes which exhibit mitochondrial and cytoplasmic forms, determined by separate loci, may provide other examples of stereochemical constraints. Presumably, the gross structural difference between SOD_S and SOD_M—one a dimer, the other a tetramer—prevents the formation of a cytoplasmic-mitochondrial hybrid form. But the reasons why other multimeric enzymes with a similar subcellular distribution (for example, malate dehydrogenase, aspartate aminotransferase, malic enzyme) do not exhibit cytoplasmic-mitochondrial hybrid isozymes, are obscure. In most cases, these isozymes have the same subunit structure and in at least one case (aspartate aminotransferase), a fair degree (about 48%) of homology in amino acid sequence^{15,16}. Perhaps the hybrid forms occur *in vivo* but are dissociated *in vitro*.

We thank Anthony Baker for technical help.

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Received 19 July; accepted 3 November 1977.

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N-methylphenylalanine at the N-terminus of pilin isolated from *Pseudomonas aeruginosa* K.

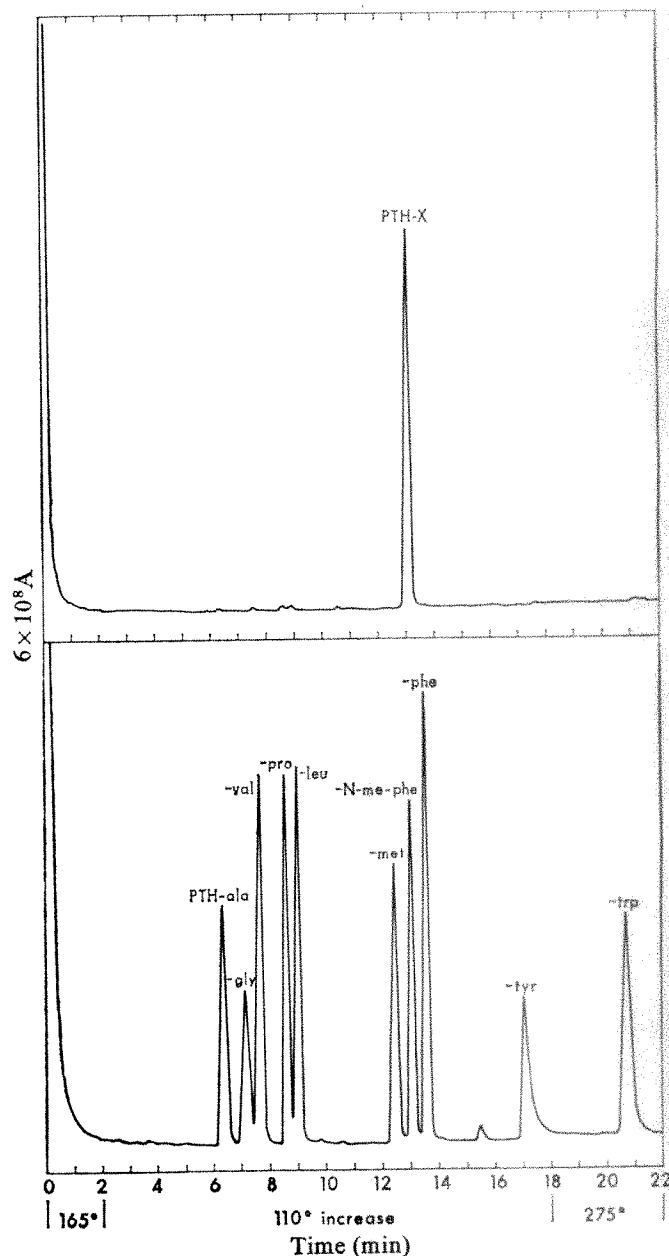
AUTOMATED amino acid sequence analysis of pili protein isolated from the multi-piliated 2PfS mutant of *Pseudomonas aeruginosa* strain K (PAK)¹ has shown that *N*-methylphenylalanine is the amino terminus. These pili, which mediate the infectious process of several *Pseudomonas* bacteriophages², are composed of one protein subunit, pilin, of molecular weight 17,800 (ref. 1). Mass spectroscopy (MS), 270 MHz proton magnetic resonance spectroscopy (PMR), ultraviolet absorption spectroscopy, gas/liquid chromatography (GLC) and thin layer chromatography (TLC) have demonstrated the identity of the phenylthiohydantoin (PTH) derivative of the amino terminal amino acid (PTH-X) to be identical with synthetic PTH-*N*-methylphenylalanine.

N-methylphenylalanine was synthesised by methylation of tertiary butyloxycarbonyl-phenylalanine dicyclohexylamine salt with methyl iodide in tetrahydrofuran in the presence of sodium hydride for 24 h at 21 °C (ref. 3), with subsequent removal of the carboxyl- and amino-protecting groups by treatment with aqueous citric acid, pH 3, and 50% (v/v) trifluoroacetic acid/dichloromethane, respectively. Reaction of *N*-methyl-

phenylalanine (90 mg) with 5% (v/v) phenylisothiocyanate in pyridine (2.4 ml) at 55 °C for 1 h gave the phenylthiocarbamyl derivative, which after drying in a vacuum, was converted to the PTH derivative by reaction with 1 M HCl (1 ml) at 80 °C for 10 min, the crude product being obtained by extraction with ethyl acetate (3 × 1 ml). Recrystallisation from diethyl ether afforded colourless crystals m.p. 113 °C.

Since the PTH-*N*-methylphenylalanine synthesised coeluted as a single peak on GLC with PTH-X, the product of the first Edman degradation of PAK/2PfS pilin (Fig. 1), and cochromatographed as a single spot on silica gel TLC (E. Merck, precoated) using two solvent systems (*R_f* 0.94 with 1,2-ethanedichloride:acetic acid 30:7 (v/v)⁴ and *R_f* 0.33 with chloroform:ethanol, 98:2 (v/v)⁵), MS, PMR and ultraviolet absorption spectroscopy were carried out to compare the two compounds further. To obtain sufficient PTH-X for PMR analysis

Fig. 1 GLC of (a) PTH-X (2% of the product from the first Edman degradation of 5 mg (280 nmol) pilin, and (b) PTH-*N*-methylphenylalanine, together with a number of standard PTH-amino acids (5 nmol of each). Separations were made on a Beckman GC-45 gas chromatograph fitted with a 1.2 m × 2 mm internal diameter column of 10% SP-400 on 100-120 mesh Supelcoport (Supelco), using helium carrier gas at 40 ml min⁻¹.



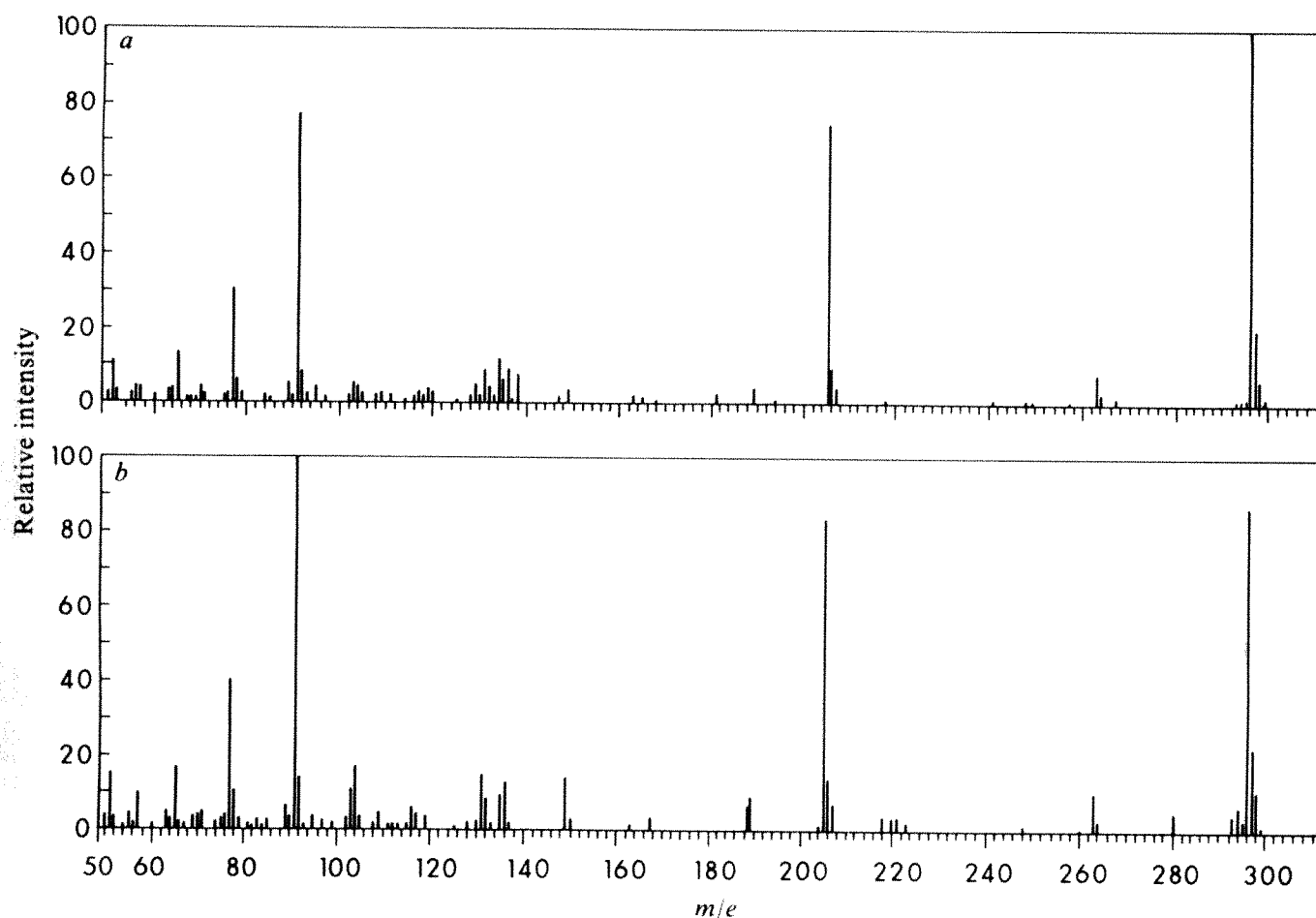
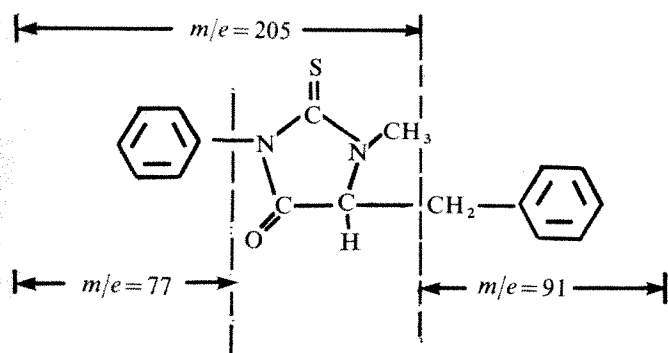


Fig. 2 MS of PTH-*N*-methylphenylalanine (a) and PTH-X (b). Both spectra were obtained on 10 μ g or less of sample by direct probe, 70 eV electron impact ionisation at an initial ion source temperature of 150 °C, using an AEI MS50 high resolution mass spectrometer in conjunction with a DS50 computer.

(0.2 mg), a combined total of 35 mg (2 μ mol) pilin were subjected to one Edman degradative cycle.

The MS of the two compounds is shown in Fig. 2. A molecular ion of $m/e = 296$ and major fragments of $m/e = 205$, 91 and 77 were evident in both spectra.



Similarly, Fig. 3 provides a comparison of the compounds' PMR spectra. The peaks downfield from 6.7 to 7.4 p.p.m. represent the two phenyl rings in the PTH derivative. The peak at 4.45 p.p.m. is due to the α -carbon proton, whereas the peak at 3.33 p.p.m. is due to the β -carbon protons. The singlet at 3.42 p.p.m. is indicative of the *N*-methyl protons.

Ultraviolet absorption spectra of the compounds showed that both have a high $\epsilon_{245}/\epsilon_{269}$ ratio of 0.64 compared with values at about 0.4 for PTH derivatives of other amino acids, with the exceptions of 0.72 for PTH-*N*-phenylthiocarbonyl-lysine and

0.67 for PTH-proline⁴. In addition, the ϵ_{269} molar absorption coefficient for the synthetic compound is lower at 13,500 than the usual value of around 16,000 for these amino acid derivatives⁴.

Attempts to regenerate and detect by amino acid analysis *N*-methylphenylalanine by hydrolysis of PTH-X and the synthetic derivative were unsuccessful. Neither acidic hydrolysis with 5.7 M HCl at 110 °C for 24 h or 57% HI at 127 °C for 20 h, nor alkaline hydrolysis with 0.2 M NaOH/0.1 M Na₂S₂O₄ at 127 °C for 4 h (ref. 6) yielded *N*-methylphenylalanine. To be able to detect this amino acid the method of Coggins and Benoiton⁷ in which a halved buffer and ninhydrin flow through the reaction coil, resulting in a double reaction time with ninhydrin, was used.

Whereas *N*-methylphenylalanine is a novel component of protein, it has been shown to be a constituent of the peptide antibiotic Staphylomycin S, produced by *Streptomyces virginiae*⁸. Pettigrew and Smith⁹ have reported the finding of dimethylproline as a blocking group at the amino terminus of *Crithidia oncopelti* cytochrome C557 and speculated on the possibility of other α -*N*-methylated amino acids existing in proteins. In the case of PAK/2Pfs pilin, however, the *N*-methylphenylalanine did not act as a blocking group to sequence analysis but merely presented a new PTH amino acid, which was not readily identified. Edman degradation in subsequent cycles proceeded normally with no obvious drop in efficiency between the first and second cycles. This is not surprising, since proline, the only other secondary amine usually encountered in protein sequence work, is not known to interfere with the process. (These sequence data are beyond the scope of this communication and will be presented separately.)

Finally, although conventional amino acid analysis of the 24-h 5.7 M HCl protein hydrolysate failed to detect the new amino

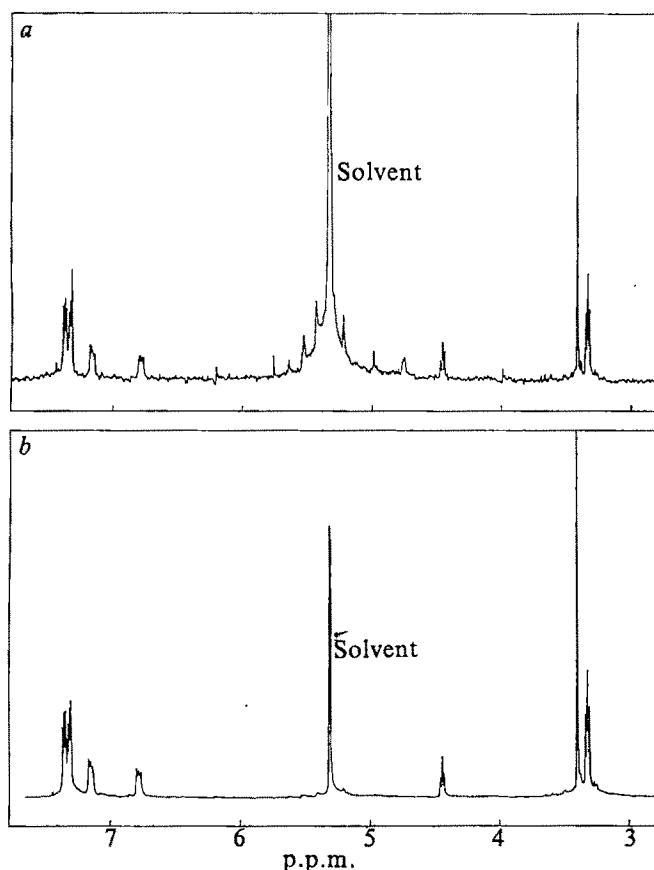


Fig. 3 PMR spectra of PTH-X (a) and PTH-N-methylphenylalanine (b). Both samples, 0.2 and 1.0 mg respectively, were washed with 99.8% D₂O and dried *in vacuo* (twice) before being dissolved in 0.4 ml CD₂Cl₂-d₂. The spectra were recorded with a Bruker HX 270 superconducting NMR spectrometer at 270 MHz and ambient temperature. Comparative peaks were adjusted to similar height. Chemical shifts are reported in p.p.m. downfield from tetramethylsilane.

acid¹, application of the method of Coggins and Benoiton⁷ permitted the detection of 1 mol *N*-methylphenylalanine per 12.6 mol leucine. Thus, on the basis of the previously reported amino acid analysis¹, which established 14 leucine residues per polypeptide (molecular weight 17,800), it is clear there is only one *N*-methylphenylalanine per polypeptide and, as already demonstrated, it occupies the amino terminus of the molecule.

This work was supported by the MRC of Canada. We thank R. S. Hodges, R. U. Lemieux and L. B. Smillie for discussions and for the use of their facilities (L.B.S.). We also acknowledge the technical assistance and expertise of M. Nattriss for amino acid analyses, B. D. Sykes for PMR spectra and A. Hogg for mass spectra.

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Chaos in the Zhabotinskii reaction

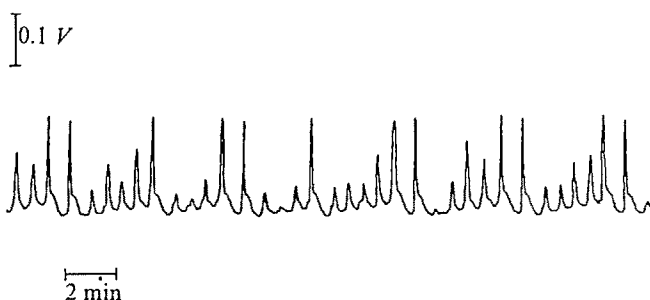
THE Belousov–Zhabotinskii reaction is a chemical Bonhoeffer–van der Pol circuit, that is, a relaxation oscillator that can be run as both an astable and a monostable ‘flip-flop’^{1–3}. Apparently the reaction also belongs to the slightly more complicated class of ‘universal circuits’⁴ as introduced by Khaikin^{5,6}. Oscillators of this type not only show ‘smooth’ and ‘relaxation type’ oscillations^{5,6}, but also ‘chaotic’ oscillations⁴. As evidenced by the simplest equation of this type⁷, both ‘spiral type’ and ‘screw type’ chaos²⁰ are possible in such systems. We present here preliminary evidence for the occurrence of screw-type chaos in the Zhabotinskii reaction.

The system was investigated in isothermal, continuous stirred flow conditions. The initial concentration of reactants was 1.8 mM of MnSO₄, 5.6 mM H₂SO₄, 0.36 M malic acid, and 45 mM potassium bromate. The volume of the reaction mixture was 36 ml. Specifically, the reaction mixture contained 50 parts of a solution ‘I’ (containing 0.34 g MnSO₄·H₂O, 153 g H₂SO₄ and 54 g malic acid l^{–1}) and 6 parts of a solution ‘II’ (70 g KBrO₃ l^{–1}). The two solutions were continuously injected by means of a peristaltic pump (Pharmacia P–3) at the same (50:6) ratio; total flow rate was 1 ml min^{–1}. The temperature was kept at 30 °C (±0.01). The volume was kept constant by means of an overflow mechanism. The electrochemical potential was measured by means of a platinum and an Ag/AgCl electrode. The system was allowed 15 min to reach a ‘stationary’ regime. A subsequent recording over 25 min is presented in Fig. 1. Similar recordings were obtained over several successive hours.

The Zhabotinskii reaction is an (about) 15-variable reaction system^{8–11}. The electrochemical potential is not uniquely related to one of these variables⁹. Only a limited amount of information can, therefore, be gained from a single measured curve. All that can be said is that the reaction is not periodic over the time interval shown (and over any time interval measured so far). If the observed oscillation corresponds to a limit cycle, the latter is bound to be of ‘complicated’ type. A complicated limit cycle (being multiply folded in state space) is, however, in itself an indicator for existing chaos. This is because the presence of such a limit cycle in most cases implies the very ‘folding’ of a cross-section through the trajectorial flow (see refs 4, 7, 20) that is also sufficient for truly non-periodic behaviour¹².

In realistic systems, exogenous perturbations are unavoidable. This means that a limit cycle of large periodicity will not be detectable if its ‘region of dominating influence’ (where the temporal behaviour is similar to that on the limit cycle) in state space is small. For then very small perturbations (for example, even the small errors of a highly accurate numerical integration routine) suffice to prevent the system from locking into the periodic regime. The longer the period, the greater this sensitivity to perturbations. On the other hand, a very large ‘pseudo-random’ period (possessing perhaps 10¹⁰ intermediate maxima,

Fig. 1 Irregular oscillation of electrochemical potential in the Zhabotinskii reaction in isothermal continuous stirred flow conditions. *V*, electrochemical potential is plotted against *t*, time.



which is not unrealistic) is, for all practical purposes, equivalent to a genuinely non-periodic oscillation.

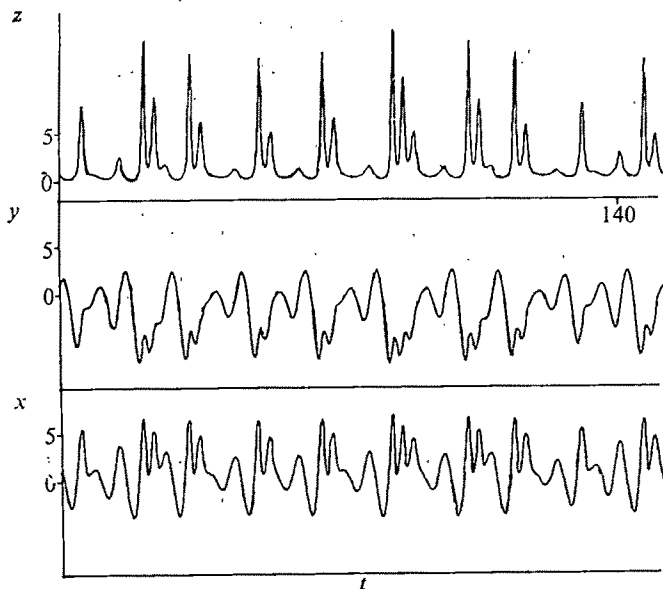
If there is exogenous 'noise' in an apparently chaotic system, the only way to detect an intrinsic chaotic component is through looking at the trajectorial flow in state space. If the flow, although being 'blurred', remains roughly confined to a sub-manifold of detectable shape, and if this shape is one of a few basic types²⁰ known to be chaos-producing in deterministic conditions, then it is possible that the system is also capable of purely 'endogenous' chaos.

Sometimes a single measured variable (or combination of variables, respectively) suffices for this proof. This is the case in spiral-type chaos⁴, for example, if the variable with the largest amplitude is the one that is measured. If subsequent recorded maxima ('amplitudes') are then plotted, each as a function of the respective last, a Li-Yorke-type map is obtained which indicates chaos^{12,13}. Other sub-manifolds in state space (like that determining screw-type chaos^{7,20}) are not so easily detectable, however. The next step in the present investigation will therefore be to simultaneously record several observables, so that three-dimensional stereoscopic pictures of trajectorial flow in observation space can be obtained.

The simplest screw-type chaotic flow⁷ has the temporal behaviour shown in Fig. 2. The time course of the first variable is reminiscent of the flow of Fig. 1. This allows the prediction that typical screw-type chaos can be found in the Zhabotinskii reaction.

The first evidence that the Zhabotinskii reaction is an oscillator of the universal circuit type was provided by the finding of 'double oscillations' in continuous stirred flow conditions^{14,15}. At that time, the dynamical implications of this result were still unknown, however. Later, A. T. Winfree (personal communication) suggested that his observation of a 'meandering' core in a non-stirred excitable version of the Z reagent¹⁶ was evidence for chaos. In the meantime, a similar phenomenon has been found in computer studies of a two-variable excitable medium (ref. 17, Rössler and Kahlert, in preparation); while two variables are sufficient for chaos in non-stirred reaction systems¹⁷⁻¹⁹, three variables are necessary in well-stirred systems. The finding that 'non-planarity' of a limit cycle in three- or higher-dimensional state space (so that a 'folded over' regime is possible) is all that is required for chaos⁴ immediately suggested that virtually all realistic chemical oscillators should be capable of chaos. Olsen has found evidence for spiral chaos

Fig. 2 Time behaviour of the three variables of the simplest screw-type chaotic oscillator. Numerical simulation of the following system of three coupled ordinary differential equations: $\dot{x} = -y - z$, $\dot{y} = x + 0.55y$, $\dot{z} = 2 - 4z + xz$. Initial conditions: $x(0) = y(0) = z(0) = 1$. $t_{\text{end}} = 152$. For a stereoscopic picture of the same flow see ref. 20.



in the horse-radish peroxidase reaction^{21,22}. Hudson's group, who had done many experiments on the Zhabotinskii reaction in continuous stirred flow conditions²³, also took a second look and found evidence of chaos²⁴. They interpreted their result in terms of the chaotic trajectorial flow described in ref. 4 which corresponds to spiral-type chaos. The present evidence for screw-type chaos (first communicated in ref. 25) does not preclude the possibility of spiral-type chaos in the Zhabotinskii reaction. On the contrary: in abstract systems, the observation of either screw- or spiral-type chaos virtually guarantees that the other type is also possible with slightly changed parameter values²⁰.

We thank Art Winfree for discussions.

Note added in proof: Two-dimensional plots (electrochemical potential against potential of a bromide ion sensitive electrode) obtained in the meantime range from the picture of a fluctuation-triggered monoflop (pseudochaos) to a picture looking like a two-dimensional projection of screw-type chaos.

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Erratum

In the letter 'Surge activity on the Barnes Ice Cap' by G. Holdsworth, *Nature* 269, p. 588, in paragraph 5 line 32 for computation¹⁰ read computation¹¹; in line 40 for balance¹⁰ read balance¹³. In paragraph 8 line 3 for form³ read form¹¹.

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matters arising

The Thera eruption and Late Minoan-IB destruction on Crete

PICHLER and Schiering¹ argue that there is no relationship between the paroxysmal volcanic eruption of Santorini in the Bronze Age and the Late Minoan-IB destructions on Crete, as proposed by Marinatos². The arguments for or against such a relationship are not as clear as they contend. There are several suggestions in their paper which may be disputed and they have been selective in their evidence. Contrary to their major point, the resolution of the pottery chronology is not sufficiently precise to infer a 50-yr gap between the destructions on Crete and the main eruption. The complexities of the relationship between IA and IB pottery styles, recently reviewed by Luce³, have been ignored. There is evidence that the two styles are in part synchronous³ and there is considerable doubt as to their stratigraphic distinction³⁻⁵.

Acrotiri was originally destroyed by earthquakes and evacuated before the eruption^{6,7} and was then apparently revisited by a group reconstructing the town, but was never reinhabited⁷. The gap between the earthquakes and the eruption is often quoted as less than two years^{1,3,5}, but this is speculation. The evidence on Santorini does not preclude a series of plausible events such as precursory earthquakes and even small eruptions, which may have discouraged reoccupation for an extended period. We agree with Pichler and Schiering that the eruption was probably very short, but challenge their interpretation of the deposits on Santorini. The occurrence of 3m lithic blocks is not evidence of the onset of caldera collapse, or even circular or polygonal fractures. There are several alternative interpretations, including ejection of lithic blocks during phreatomagmatic explosions, blocks eroded from the vent wall, blocks picked up by mud-flows or pyroclastic flows from the volcano's slopes⁸. The largest lithic blocks on Santorini are predominantly found in deposits interpreted by Bond and Sparks⁸ as mud-flow not ash-flow deposit. Bond and Sparks⁸ also describe late-stage flood deposits and ignimbrites which were emplaced before substantial collapse had begun. A gap of unknown duration between the eruption

and caldera collapse cannot be discounted.

The confidence with which Pichler and Schiering assert that the ash layer was of negligible thickness on Crete is unwarranted. Ninkovich and Heezen⁹ provided firm evidence of the large scale of the eruption and of a high probability of ash-fall on Crete. Their evidence cannot be discarded, as later papers have confirmed the correlation of the Minoan ash layer^{10,11} in RV Vema cores from the Lamont-Doherty Geological Observatory by the presence of the 7,000-yr BP sapropel and chemical analyses. Although the ash layers in cores V10-50 and V10-58 are undoubtedly overthickened by slumping, a reasonable thickness of ash has to accumulate initially to produce a slump. Pichler and Schiering claim to have succeeded in the most difficult of geological tasks, to show that visible remnants of the Minoan ash do not exist on Crete. Their dismissal of the presence of volcanic glass fragments in Cretan soils¹²⁻¹⁴ as due to the use of imported pumice lumps in Minoan households and workshops is unconvincing, and we suggest that this evidence supports ash-fall on Crete. Similarly, to dismiss the possibility of catastrophic tsunamis on the basis of an unpublished theory is unacceptable. Finally we note that the 'isoseismic' data in their Fig. 2 concerning the 1926 earthquake, published over 40 yr ago, seems to be a function of the density of population and hence observation points as well as possible tectonic blocks. We believe, therefore, that more evidence is required to test the theory of Marinatos² relating the Minoan demise to volcanic activity. There

are three time gaps to be considered: that between the earthquake(s) which destroyed Acrotiri and the onset of the eruption, that between the destruction of Acrotiri and devastation of Crete and that between the eruption and caldera collapse, and possible resulting tsunamis. The many uncertainties in the length of these time intervals is such that the volcanic theory cannot be discarded.

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Possible solar eclipse effect 23 October 1976

LILLEY and Woods¹ operated magnetometers at 10 sites in central and eastern Australia during the solar eclipse of 23 October 1976, and reported a possible eclipse effect at two stations (labelled H and J in their Fig. 1) lying in the path of totality. They observed an event at approximately the time of totality (0638 UT) but pointed out that the event had not moved from west to east at the expected rate. As an eclipse affects the ionosphere over a relatively large area one would not expect the few minutes of totality to produce such a pronounced effect as indicated by their records.

In South Africa the eclipse was partial and of limited duration, starting at sunrise (~ 0420 UT). The records of the magnetic observatories Hermanus (34°S, 19°E) and Grahamstown (33°S, 27°E) should be free of eclipse effects at the time of totality in Australia (0638 UT). The declination variations as recorded at these two observatories over the period illustrated by Lilley and Woods are shown in Fig. 1. It is evident

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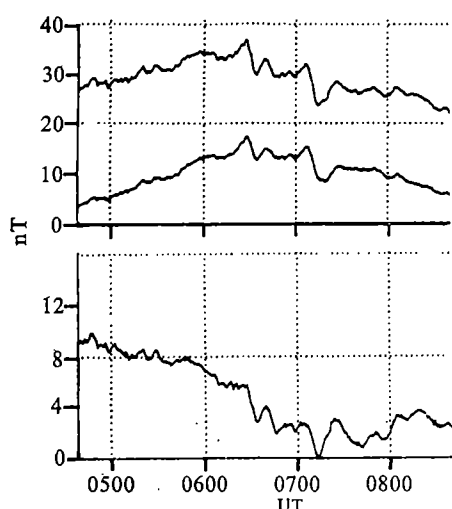


Fig. 1 The declination records for Grahamstown and Hermanus, and the Grahamstown-Hermanus difference curve, plotted on the same scale as used by Lilley and Woods¹. All records are plotted relative to arbitrary zeros.

that the magnetic field in South Africa was slightly disturbed during the interval 0620–0640 UT when the eclipse occurred in Australia. The 0711 event mentioned by Lilley and Woods is also present although the relative amplitude is smaller than in Australia. The Grahamstown-Hermanus difference graph shows a rapid variation at 0628 UT compared to 0638 UT in Australia. The event reported by Lilley and Woods could, therefore, have been a small eastward travelling disturbance. One should, however, be careful not to attribute all sudden changes in the difference curves to a phase shift. High frequency variations at site J are clearly more damped than the corresponding variations at site H. The form and smoothness of the difference curves are in fact determined to a large extent by the relative frequency responses at the two sites, and not by phase shifts, except in the case of the S_q variations. Similar effects can be seen in the Hermanus-Grahamstown comparison. High frequency response at Hermanus is markedly lower than at Grahamstown.

We conclude that the event reported by Lilley and Woods is a manifestation of a minor magnetic disturbance. The variations observed at the Gngangara observatory in Western Australia should give more information on the suggested eastward travelling event.

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LILLEY AND WOODS REPLY—We accept Scheepers' evidence for some widespread minor magnetic activity at the time of the eclipse in south-east Australia. If the source currents for this activity flowed in the overhead ionosphere they (like the S_q currents) may be perturbed by lowered ionospheric conductivity in an eclipse shadow, and we are now examining the

data from our line of instruments for such an effect. We agree with Scheepers that such an ionospheric effect may be difficult to distinguish from local differential induction.

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Climatic interpretation of $\delta^{18}\text{O}$ and δD in tree rings

THERE has been considerable interest^{1–5} in, and some controversy^{5–7} over the use of the stable isotopes of oxygen and hydrogen in tree rings as measures of past climate. We point out here that variations in isotopic values from the whole ring (or a number of rings) may be in part a function of variations in ring width. As a consequence, whole ring isotope estimates of past climate will not be independent of ring width estimates of past climate. This is a direct result of the relative constancy of either early- or late-wood width in many tree species and of the differences in isotopic composition between early and late wood.

In many species of tree, much of the variation in ring width from year to year is in either the early-wood or the late-wood part of the ring. For instance, in oak⁸ and elm (D. Brett, personal communication) the width of the early wood is approximately constant, while in most conifers the late-wood width is approximately constant⁴. Early wood and late wood generally have different isotopic composition. The cellulose analyses of Epstein and Yapp⁵ and Wilson and Grinsted⁴ both show differences of up to 50‰ in δD . The latter authors show lighter isotopic values in the early wood which they attribute to temperature-dependent fractionation effects. Epstein and Yapp⁵ disagree with this interpretation. Their analyses show that early-wood isotopic values may be either lighter or heavier than those of the late-wood depending on the location of the tree, and their work suggests that late-wood is isotopically lighter (for Douglas Fir) in regions where there is winter snow cover.

Epstein and Yapp⁵ note that "... differences in early- and late-wood δD could cause sampling problems...". We elaborate here on one way which such problems might arise, and on their possible implications. Approximate constancy of early- or late-wood width, and isotopic variation across a ring, when considered together, necessitate a relationship between ring width and the isotopic value ($\delta^{18}\text{O}$ or δD) of the whole ring. If E is the early-wood width, L the late-wood and $W (= E+L)$ the total ring width, and if δ_E , δ_L and δ_w are the corresponding mean stable isotope

($\delta^{18}\text{O}$ or δD) values, then

$$\delta_w = \delta_E + (\delta_L - \delta_E) L/W \quad (1)$$

or

$$\delta_w = \delta_L - (\delta_L - \delta_E) E/W \quad (2)$$

These alternative expressions for δ_w arise from the fact that δ_w is a weighted average of δ_E and δ_L . They both contain a term proportional to W^{-1} and thus imply a direct functional dependence of δ_w on W . The sign of the relation between δ_w and W depends on the sign of $\delta_L - \delta_E$ and on whether the tree has relatively constant E or relatively constant L .

As ring width is determined partly by climate (although the relation is often complex⁹), these results imply a dependence of δ_w on climate which would occur even if δ_E and δ_L were constants. Thus, at least a part of any δ_w -climate relationship may be attributable to ring width variations.

Whether this 'ring width effect' contributes significantly to variations in δ_w depends on the magnitude of variations in δ_E , δ_L and either E/W or L/W . For oak, where E is approximately constant and equation (2) is the appropriate expression, Eckstein and Schmidt⁸ give ring width data where E/W varies from 0.23 to 0.63 over the period 1880 to 1969. Since $\delta_L - \delta_E$ may be as high as 50‰ for δD , equation (2) shows that the ring width effect could give δD variations for single whole rings of up to 20‰.

Expected variations in $\delta^{18}\text{O}$ of whole rings due to the ring width effect are difficult to estimate because no suitable early-wood and late-wood isotopic data is available. The values would depend on whether precipitation or temperature-dependent fractionation effects are dominant in controlling tree ring isotopic composition, a subject on which there is considerable disagreement. If precipitation effects dominate then $\delta^{18}\text{O}$ variations of order 2.5‰ could be expected as a result of the ring-width effect. If fractionation effects dominated then even greater variations in $\delta^{18}\text{O}$ could occur.

Gray and Thompson¹ and Libby *et al.*² have found significant correlations between whole ring isotopic values and mean annual temperatures. Their results may be partly due to the ring width effect. Gray and Thompson's¹ data show a $\delta^{18}\text{O}$ range of about 3‰ with lower values corresponding to cooler mean annual temperatures. In their case we expect L to be relatively constant. If $\delta_L - \delta_E < 0$, as suggested by the results of Epstein and Yapp⁵, then δ_w should be lower for narrower rings (see equation (1)), and hence, most probably, for colder years. Since Gray and Thompson use 5-yr means, the magnitude of variations due to the ring-width effect could be as much as 1.5‰.

and could account for a significant part of the variation observed. If $\delta_L - \delta_E > 0$ then the ring-width effect would be in the opposite direction and the variation observed by Gray and Thompson¹ would be even more significant.

The results of Libby *et al.*² are more difficult to interpret. One of their oak samples shows variations in δD and $\delta^{18}O$ of approximately 180‰ and 9‰ respectively (for 30-yr means). This variability is much larger than one would expect from the ring-width effect. As Epstein and Yapp⁶ point out, however, the magnitudes of the variations observed by Libby *et al.*² are much larger than one would expect from precipitation isotope variations (which Libby *et al.*² state to be the dominant control). Such large variability is even more unexpected when one recalls that Libby *et al.*² use 30-yr means. Nevertheless, their results do correlate reasonably well with central England temperature data.

Isotopic data from tree rings are potentially a powerful indicator of past climate. There is, however, considerable disagreement between the opinions of different workers in this field, and many of the results seem to be mutually incompatible. The simple analysis presented here suggests that variations in whole ring isotope data may be significantly influenced by variations in ring width, and that such isotope data need not be an independent climate indicator. This additional complicating factor may help to explain some of the incompatibilities. We suggest that a comparison between ring width and isotopic variations should be a first step in any analysis of whole ring isotopic data, whether the data comes from whole wood or chemically more specific isotope measurements.

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WILSON REPLIES—Wigley, Gray and Kelly make the valid point that isotopic measurements on whole wood may give results which merely reflect the ring width. One aspect of the problem was discussed by Wilson and Grinsted¹ who pointed out that whole wood contains a mixture of lignin and cellulose which have different isotope compositions. Since

early and late wood can have different lignin to cellulose ratios, isotopic measurements on whole wood may merely reflect the ratio of late wood to early wood which may in turn be a simple reflection of ring width. Wilson and Grinsted suggested that this and other problems could be avoided if isotope dendroclimatologists measured only pure cellulose or pure lignin.

The comments of Wigley *et al.* on the climatic interpretation of the isotopic measurements on tree rings and the relationship of these to past climate involve the fundamental principles on which isotope dendroclimatology is based. Much of the controversy in this field stems from certain misconceptions of the aims of isotope dendroclimatology and the basic plant physiology involved.

Isotopic dendroclimatology studies face two problems. First, at what times of the year is the material that is ultimately formed into wood components fixed from the atmosphere? And second, how does the isotopic composition of a wood component such as cellulose vary with changing climate?

A conifer manufactures photosynthates at all times of the year except when climatic conditions are unsuitable, due for example to low temperatures or drought stress. These photosynthates are stored for short or extended periods before they are laid down as wood. Most conifers (*Pinus radiata* is one of the exceptions) lay down wood only during a brief period of the year. The actual period of wood deposition is not controlled by net photosynthesis but is under hormone control, the production of hormones being controlled principally by day length and to a lesser extent by temperature. For example, in trees suffering from drought stress wood can be laid down during periods when net photosynthesis is negative².

The above considerations apply to any isotopic work on trees. But in the case of isotopic work on oxygen and hydrogen another factor is important. The isotopic composition of rain or snow depends on many factors including the temperature history of the air masses which bring the precipitation to an area³. This is not the end of the problem, however. Once the water is taken into the tree transpiration processes in the leaves can cause very large fractionations particularly in arid environments⁴.

Wigley *et al.* state that “early wood and late wood generally have different isotopic composition”. Leaving aside the point, discussed above, that early and late wood generally have different D/H ratios because they are a variable mixture of more than one compound each with its own isotopic composition. Late-wood cellulose and early-wood cellulose generally have different isotope composition because they represent material fixed from the atmosphere at different

times of the year and hence in different climatic conditions. For example in New Zealand *Pinus radiata* early wood is laid down in the spring and summer and late wood in the winter, that is, early wood is laid down at a warmer time than the late wood. On Mt Lemon, Arizona, *Pinus ponderosa* lays down early wood in the spring and late wood in the summer⁵. That is, early wood is laid down at the colder time than the late wood. The important point is that the isotopic composition is determined by the climate at the time the carbohydrate was fixed from the atmosphere and not whether it is early or late wood that is being formed.

Isotope dendroclimatology can never be expected to produce a mean annual temperature curve as might be produced by a meteorological observer or from measurements of speleothems⁵. It will only produce a curve representative of some period of the year for example ‘a spring and early summer’ temperature curve. Different trees may fix from the atmosphere the carbohydrate ultimately formed into wood at different times of the year and hence record climate at different periods of the year. Obviously a deciduous stress such as an oak growing in New Zealand only carries out photosynthesis in the spring and summer, whereas at the same site a conifer such as *Pinus radiata* can fix material from the atmosphere all times of the year. Thus the wealth of palaeoclimatic data recorded in the isotopic ratios of the constituents of tree rings and the superb time base provided by dendrochronology makes the potential of isotopic dendroclimatology very great indeed.

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GRAY AND THOMPSON REPLY—The ‘ring width effect’ discussed by Wigley *et al.* is relevant in two regards to our findings of significant correlation between $\delta^{18}O$ values in cellulose and mean annual temperatures¹. First, as stated by Wigley *et al.*, there is a possibility that an appreciable fraction of the variation in the $\delta^{18}O$ values may be due to varying proportions of early and late wood in the tree rings used for analysis. Second, the possibility exists of a sampling error being introduced when a 5-yr group of rings is analysed.

To evaluate the first effect we have made measurements of late-wood width (*L*), early-wood width (*E*), and total ring

width (W) for the entire section of tree used for production of the climate curve¹. Values of L/W ranged from 0.05 for the largest value of W to 0.32 for the smallest W . Further, $^{18}\text{O}/^{16}\text{O}$ isotopic analyses were carried out on early and late wood from a number of selected rings from the tree. (These were necessarily rings having larger values for W due to difficulties associated with the sampling of narrow rings and insufficiency of material for analysis.) The range of values found for the difference in isotopic composition between early and late wood ($\delta E - \delta L$) was found to be 0.5 to 0.8‰.

Thus using equation (1) as stated by Wigley *et al.* with values of $\delta L - \delta E = -0.8$ ‰ and $L/W = 0.32$ the maximum variation in δW due to the term dependent on total ring width is found to be 0.26‰. Thus the maximum contribution to the variation is estimated at somewhat less than 10% of the total variation observed (3‰) in a 100-yr-old tree. Furthermore, by taking 5-yr groups of rings this effect will tend to be minimised since the tree in question contained few narrow rings and the effect is much less significant for wider rings (L/W of the order 0.05). In general, therefore, we expect the effect to be considerably less than the 10% suggested above.

The measured values of tree ring width (W) for the Edmonton spruce, after growth curve corrections were made, show little or no correlation with mean annual temperatures. We conclude that in this case, the contribution of ring-width effects to variation in $\delta^{18}\text{O}$ of the cellulose is minimal. This finding, however, does not detract from the potential significance of the effect for other trees in other climate zones. Care should be taken to select trees which are 'complacent', that is, those with ring widths showing little annual variation. (This is in contrast to the requirements of those establishing climate curves from ring-width measurements².) The second possible effect of ring-width variation, that of introduction of a sampling error, arises because, when sampling 5-yr groups of rings¹, no attempt was made to normalise the amount of wood contributed by each of the rings to the material subsequently analysed. When calculating mean annual temperatures for each 5-yr period, however, an arithmetic mean was used, implying equal contribution from each ring, and hence implying equal ring width throughout the 5-yr group. To estimate the error introduced by this procedure, we measured total ring widths for the entire tree. Using mean annual temperature (T) from climate records we calculated the expected isotopic composition of the cellulose in each ring using the relation $\delta^{18}\text{O} = 1.3T + 20.5$ (‰) (ref. 1). The mean $\delta^{18}\text{O}$ for each 5-yr group of rings was then calculated first using

Table 1 Comparison of ring-width weighted mean, non-weighted mean and measured $\delta^{18}\text{O}$ values for cellulose extracted from 5-yr groups of tree rings

Period	$\delta^{18}\text{O}$ (‰) weighted mean	$\delta^{18}\text{O}$ (‰) unweighted mean	$\delta^{18}\text{O}$ (‰) measured
1894-99	23.4	23.5	23.4
1900-04	24.2	24.4	24.1
1905-09	24.7	24.6	24.7
1910-14	24.6	24.6	24.7
1915-19	24.0	24.0	24.1
1920-24	23.8	24.0	24.2
1925-29	23.7	23.5	23.6
1930-34	24.6	24.7	24.7
1935-39	23.2	23.3	22.9
1940-44	24.9	24.6	24.5
1945-49	23.8	23.7	23.3
1950-54	23.7	23.3	23.3
1955-59	24.4	24.5	24.5
1960-64	25.1	25.3	25.2
1965-68	23.5	23.7	24.0

the ring-width data as a means of weighting the $\delta^{18}\text{O}$ contribution of each ring and second, assuming equal contributions for each ring in a given 5-yr group. The calculated weighted and unweighted means, together with measured values are shown in Table 1. The largest difference between weighted and unweighted calculated value is 0.4‰, approximately twice the estimated precision of the measured data (± 0.2 ‰). It seems that errors introduced elsewhere in the procedures used, are usually equal to or greater than those due to sampling errors. It should be pointed out, however, that trees showing marked variability of ring width with climate may well present sampling problems of this kind.

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LIBBY AND PANDOLFI REPLY—Pandolfi has shown that isotope variations in tree rings are correlated with climate variations local to the trees. Wigley *et al.* speculate that isotope variations in tree rings may be correlated with ring widths. Then if they are correct, it must follow that ring widths are correlated with climate.

We consider that the master chronologies of ring widths from the European tree laboratories will not correlate with local climate variations. We found no correlation between isotope measurements for a German oak and B. Huber's master oak ring chronology.

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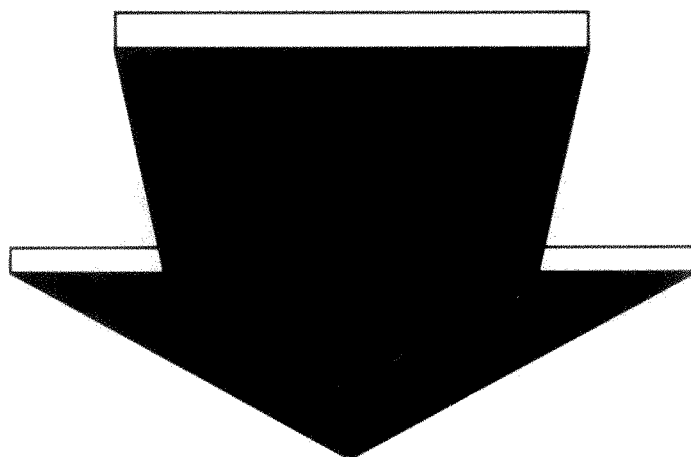
Amide nitrogen is unlikely to be a proton acceptor

In their article on the mechanism of catalysis of acid proteases and extended to proteases generally, James *et al.*¹ make several statements to the effect that a tyrosine donates its proton to the amide nitrogen of the scissile bond in a peptide substrate. This mechanistic idea is similar to that suggested for hydrolysis of peptide substrates by carboxypeptidase^{2,3}. But the peptide nitrogen is not the basic site in an amide bond and cannot accept a proton, a hydrogen bond, or a metal ion in the ground state of an enzyme-substrate complex⁴. This restriction holds with even more force when the amide carbonyl oxygen interacts with a proton or a metal ion, as is thought to be the case with carboxypeptidase.

There are many data to support the view that the basic site in an amide bond is the carbonyl oxygen so that either a proton^{5,6} or a metal ion^{7,8} associates at that atom. These conclusions are supported by theoretical calculations which indicate a strong preference for protonation of amides at the carbonyl oxygen^{9,10}. Unless there is enormous strain associated with substrate binding, the earliest stage in a hydrolysis mechanism during which an amide nitrogen may become a proton, hydrogen bond, or metal ion acceptor is concerted with nucleophilic attack at the amide carbon which results in both the carbon and nitrogen of the amide bond taking on tetrahedral character⁴. At this point the nitrogen has lost its amide character and has become an amine nitrogen. This principle seems to have been accepted in a recent discussion of the mechanism of thermolysin which is thought to be similar to that of carboxypeptidase¹¹. Though the authors of the acid protease paper¹ may feel that they did not violate this principle in their own minds, their statements coupled with proposed mechanisms in the literature cited are apt to mislead many readers.

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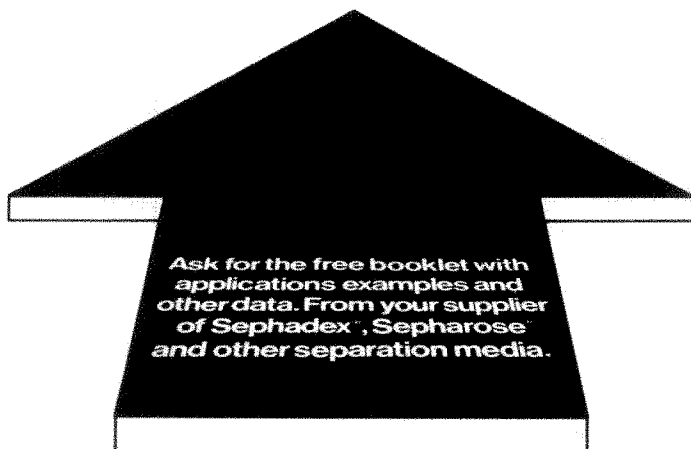
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edited by **Joan Davidson**, *Lecturer in Rural Planning, Gloucestershire College of Art and Design, Cheltenham*, and **R. Lloyd**, *Principal Research Officer, Countryside Commission*.

This book sets out some of the consequences of recent and further developments in agriculture for the landscapes and major ecosystems of the farmed countryside; but it is also concerned with the means by which wildlife and landscape values might be safeguarded. The authors have tried to be realistic, suggesting where the ecological effects of modern agriculture matter most, and exploring those means of conservation which seem to be economically and politically feasible at the present time. Throughout, the aim has been to bridge some of the gaps in information and understanding that exist between the many different groups with a stake in rural land management, including farmers and their advisers, planners, ecologists and conservationists.

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MARINE ECOLOGY Vol. 3, Parts 2 & 3

edited by **O. Kinne**, *Biologische Anstalt Helgoland, Hamburg*.

Ecology comprises studies of organisms in relation to their environment, abiotic and biotic. Marine ecology deals with the vast multiplicity of organisms living in oceans and coastal waters. This treatise attempts to cover all major aspects of marine ecology. It consists of 5 volumes. Volume III, Cultivation, reviews the information which has accumulated on our capacity for supporting marine micro-organisms, plants and animals under environmental and nutritive conditions which are, to a considerable degree, controlled. (Marine Ecology Series)

Contents: Part 2: Cultivation of Animals — Research Cultivation (O. Kinne). Part 3: Axenic Cultivation (L. Provasoli); Commercial Cultivation (Aqua-culture) (O. Kinne and H. Rosenthal); Multispecies Cultures and Microcosms (M. Lerandowsky); Chemical Contamination of Culture Media: Assessment, Avoidance and Control (M. Bernard).

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reviews

American Natural History

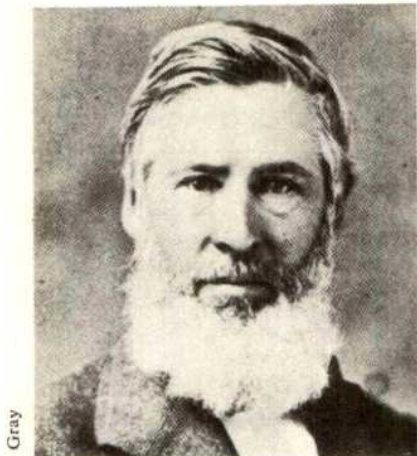
Jack Major

Natural History in America. By Wayne Hanley. Pp.xiii+334. (Harper and Row: London; Quadrangle/New York Times Books: New York, 1977.) £8.95; Fl.40.65; \$14.95.

THE author compiles and discusses excerpts from the record of natural history observations during settlement of the US over the past two centuries. Ornithology is emphasised. Colour plates of paintings made by the eighteenth- to nineteenth-century naturalists are included. Unfortunately, the book has no index, nor reference list. Hanley's method is to quote passages from the early naturalists' published work and to relate them to then contemporary events in the biological sciences at large. Thus, Linnaeus and his emissary Peter Kalm, as well as Darwin's work, are very much present in early American natural history. The author introduces his book modestly: "This is not a profound volume. It is essentially a collection of readable passages selected from the writings of great American naturalists" (page xi). Too modestly. The events and thoughts described and quoted have acquired a profundity in our time when America has used up so much of its bountiful inheritance and when so many are so concerned that some of our wonderful natural heritage be passed on to future generations.

Modern naturalists will be deeply impressed, also, by young Meriwether Lewis setting forth on his extended (1804-1806) field trip into the wilderness bearing a hand-written, open letter of credit from President Jefferson authorising unlimited funds on the credit of the US Government. The Indian woman, Sacajawea, who guided him through the Rocky Mountains was of more help.

The book opens with a chapter, "In the Beginning: Catesby", on "an exception to the general rule of incompetence or worse among early eighteenth-century naturalists"—Mark Catesby's hand-coloured, illustrated *Natural History of Carolina, Florida and the Bahama Islands* of 1730-1748. His stiff but accurate paintings are followed by discussion of John and William Bartram, collectors of plants and ornithologists; of Alexander Wilson, with



Gray

his colour-illustrated, nine-volume *American Ornithology*; and climaxed by Audobon's magnificent paintings and vivid writings and his collaboration with the mammalogist John Bachman. The western part of the present US was almost unknown to these naturalists, and one gets the impression they regarded its biological riches from the standpoint of the Boston dowager who described her route to San Francisco, over 4,000 km distance, by way of Newton, a suburb of Boston. But they were fully occupied with the area east of the Mississippi River from Labrador south to Florida, and the modern naturalist can only appreciate and envy their discoveries.

Lewis and Clark explored westward from St Louis, Missouri, to the Pacific. They are given a chapter. Hanley emphasises their written interest in the grizzly bear and Indian horses, almost to the exclusion of the other myriad new forms of plant and animal life they encountered on their epic journey. Horses are of obvious interest to anyone on a walk from St Louis to the mouth of the Columbia River. And anyone afoot, armed or unarmed, who has seen a grizzly moving ponderously but so fluidly about its own business and then throw up its head to stare at the intruder, will sympathise with Lewis and Clark's deep interest.

Although Hanley's book has in its title *America*, Canadian contributions are absent. The voyageurs who performed miracles of physical labour and daring in penetrating America toward the Pacific, and the Hudson Bay and North-West Company factors who em-



Agassiz

ployed them seem to have written little on the Natural History of these virgin lands, for reasons of illiteracy or business priorities. But Sir Alexander Mackenzie's journals describing his explorations from Lake Athabasca to the Arctic Ocean (1789) and then to the Pacific at Bella Coola (1792-1793) are not devoid of Natural History observations. True, he noted birch and spruce because they could furnish bark and gum to repair his canoe, various shrubs because their berries were edible, devils club (*Oplopanax horridum*) because it hindered his progress; but transportation and food of necessity were his primary interests. And he did hesitate on his perilous return journey to measure one of the giant cedars (*Thuja plicata*) which he noted characterised the magnificent coniferous forest of the Pacific slope.

But Hanley has plenty of material in the US alone. Thomas Nuttall collected plants throughout much of the US, wrote a catalogue of American plants, a botanical textbook, a bird manual, spent 11 years at Harvard University, and ended up on an inherited estate in England. Thomas Say, on the other hand, seems to have been totally disinterested in money, but he was interested in collecting, travelling, and he advanced Natural History knowledge, uniquely in entomology and conchology.

N. M. Hentz, too, was eccentrically interested in less well-known organisms, spiders in his case. The climax comes with Rafinesque. It was a taxonomic climax, only exceeded by the collections of the exploring expedition

around the world under Lieutenant Charles Wilkes, who not only went to Antarctica but whose shore parties contributed to our knowledge of Mount Shasta in northern California. Hanley also devotes a chapter to the whalers, including the novelist Herman Melville who was a crew member in the bloody business "until he jumped ship at Marquesas Island in July, 1842, preferring to live among cannibals".

And finally comes the first writer and naturalist in Hanley's collection who looked at nature as a whole, with sympathy. Did Thoreau's words exert much influence? Probably not, but we are discovering them anew. He is a charming prophet.

In the following chapter, Agassiz and Asa Gray differed over Darwin's ideas without adding much to them, and they gave Harvard an early taste of dissension among its faculty.

Academic nastiness accompanies Cope and Marsh in their disputes over priority of publication, ownership of specimens, money, and anything else handy one feels. Living reptiles and amphibians found a specialist in J. E. Holbrook.

Elliott Coues was an improbable cavalryman, briefly a member of the mystical Theosophical Society of India, a superb ornithologist whose description and advocacy of bird identification using a shotgun is illuminating. Hanley progresses from the "I do not protect birds, I kill them" school of ornithology to preservationist attempts to stop some of the slaughter of wildlife. First, to stop commercial use of bird skins to decorate women and, later, to save enough big game so hunting could continue. Protagonists of the latter crusade included W. T. Hornaday and Theodore Roosevelt. In the western US, John Muir took up some of the same themes Thoreau had broached in the east. He, too, was ahead of his time in suggesting that not every last resource should be used up by the generation then in propertied possession.

Popularisers of Natural History such as John Burroughs and Ernest Thompson Seton aroused interest in the life histories of mammals in general. If Seton was an egotist, he also had much field experience and was a competent mammalogist. Burroughs deliberately kept the observer (himself) in the forefront of his observations and descriptions, to the detriment of both. Louis Agassiz Fuertes seems to have been a paragon, not only as a painter of birds but as a fine field companion.

An appreciation of the place of predators in the scheme of nature began to appear only with C. Hart Merriam, working within the government. Aldo Leopold progressed to this view, wrote

a textbook which made wildlife management a field of study, and suggested a philosophy of land use in consonance with Thoreau's ideas. But their ideas are still ahead of the economic and commercial mores of our time. Our time is one of continuing pollution of our environment, and Rachel Carson has the last chilling word.

Each person will have his own list of people, quotes, ideas, landmarks, interactions left out of Hanley's little book. The early geologists such as Gilbert, Dutton, Powell, Russell in the western US, and the early ecologists such as

Forbes, Clements, Cowles, Harper, Kearney, Sears, Weaver would be mine. But even if the scope of American Natural History over the past 200 years seems occasionally to approach a Boston ornithologist-dowager's view, Hanley has produced a wonderfully readable and useful account. I hope it will stimulate readers to learn more of the background of our present ecological crises and the science that works toward their solution. □

Jack Major is Professor of Botany at the University of California at Davis.

Running a bird reserve

Minsmere: Portrait of a Bird Reserve. By Herbert Axell and Eric Hosking. Pp. 256. (Hutchinson: London, 1977.) £7.50.

BASICALLY, as its title suggests, this is the story of the setting up and running of a reserve. It is also, however, rather more than that. Minsmere, on the Suffolk coast, is one of the largest and most famous reserves run by the Royal Society for the Protection of Birds (RSPB), and the author was the warden of the reserve for 17 years and was responsible for many of the innovations carried out there.

Before the last war, Minsmere was a low-lying area of farmland, vulnerable to flooding by the sea. In June 1940, it was intentionally flooded as part of Britain's coastal defences against possible invasion by the Germans. In short, the birds approved and by the end of the war a number of rare species were established there. In 1947, the RSPB acquired a lease of the new marshes, together with adjacent areas of heath and woodland.

The book describes the birds of all the Minsmere habitats, giving particular prominence to those that are rare elsewhere in Britain. There are four of these; three—the Bittern, Marsh Harrier and Bearded Tit—are reed-bed birds. Once common over wide areas of Britain, these species have slowly dwindled with the general drainage, particularly of the Fens. The Marsh Harrier and Bittern are reduced to very small numbers in the UK at present, indeed at one stage Marsh Harriers bred nowhere apart from Minsmere. It is one of those wry complexities which nature all too frequently sets for conservationists, that anxious parent Bitterns occasionally satisfy the needs of their clamouring young with baby Marsh Harriers from an adjacent nest. The Bearded Tit is more numerous than the other species and at the

moment more widespread. It was, however, once scarcer and at that time Minsmere was the essential base from which, at a later date, the other colonists spread out.

Maintaining habitat in the stage at which the birds need it, is not just a matter of sitting about; it needs active management. This is especially the case with the fast growing reed-beds. The author not only discusses his day-to-day problems, but also describes new developments which he initiated. Over a series of winters (so the breeding birds would not be disturbed), bulldozers gradually made a series of shallow pools, now famous as "The Scrape". To these, and their gravel-covered islands, birds came in large numbers. Among these were the terns, whose nesting areas along the beach were almost ceaselessly disturbed by people; and waders, whose shallow feeding areas are now so scarce. The other bird to approve of these moves was the Avocet—the fourth species to merit a chapter to itself. With only one other nesting colony in Britain (on Havergate, another RSPB reserve), this was a welcome development.

Perhaps one of the points that emerges from this book is how successful a man-made marsh can be. One cannot, at will, reproduce a mature oakwood, but one can reproduce quite quickly many of the necessary features of a marsh. As drainage relentlessly goes on, perhaps more organisations could be persuaded to find low-lying land which could be used to make amends, albeit in a small way, for some of the losses.

The book is beautifully and profusely illustrated, mostly by Eric Hosking's own photographs. My only criticism is that some of the colour photographs are reproduced at six to a page, and the small size does not permit one to fully appreciate their quality.

C. M. Perrins

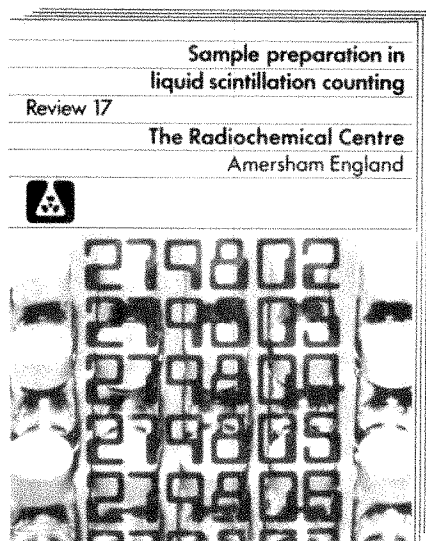
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Sokoloff, L. et al, "Mapping of Functional Neural Pathways by Autoradiographic Survey of Local Metabolic Rate with [¹⁴C] Deoxy-glucose," SCIENCE, 187, 850-853 (March 7, 1975).

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Butterfly origin

The British Butterflies: Their Origin and Establishment. By R. L. H. Dennis. Pp. xviii+318. (E. W. Classey: Faringdon, UK, 1977.) £10.

THE message of this book is simple: recent work on the British Pleistocene suggests that all Britain's butterflies must have been exterminated by cold before the amelioration of climate which began 10–15,000 yr ago; no existing populations of butterflies in the British Isles, including various endemic "subspecies", can be older than that. This view contrasts with the earlier ideas of B. P. Beirne and E. B. Ford, who, working with a radically different and now outmoded reconstruction of Pleistocene events, considered that at least some of our butterflies had existed continuously in Britain for 100,000 yr or more. Dennis's book has some subsidiary themes, one being that phenotypic divergence of a butterfly population bears no direct relationship to either its antiquity or total genetic divergence. I agree with both of these conclusions.

Dennis's treatment of the subject is based on a wide reading of the work on the Pleistocene, local literature pertaining to the distribution and ecology of British butterflies, and an eclectic selection of genetical texts. Two extremely relevant areas of work are completely ignored: MacArthur–Wilson island biogeography theory, aspects of which Dennis apparently rediscovers for himself, and Paul Ehrlich's school of work on *Euphydryas* colony structure. He does refer once to the classic paper of Ehrlich and Raven on butterfly and plant coevolution. In doing so, he misspells Erlich (*sic*), gives the wrong publication date, and apparently misunderstands the particular ideas of biochemical coevolution involved. Further, in discussing phenotypic divergence, antiquity and evolutionary rates, Dennis makes no reference to work on rates of protein evolution.

If the essential message of the book is simple, and key areas of the literature have been ignored, why does Dennis's main text span 270 close-set pages? Because he frequently uses whole sentences where one word (or even none!) would suffice. He is at pains at every point to indicate that he has thought of every possibility. He attacks the same problems again and again, using the very few lines of evidence open to him. As a result, instead of being a delightful natural history detective story, the book is bewildering. Alternative hypotheses are not presented and dealt with in a logical way, step by step. Instead, we are fed with an unending torrent of jargon-laden fact, theory and specula-

tion, leaving the reader in a state of dizzying confusion. Beneath this, many of Dennis's ideas are sound, and one gets an impression of tremendous enthusiasm. If so, it is unfortunate that the book has been published in its present, clearly unedited state, because both the author and his intended audience are the losers.

In short, although it will have some value as a reference source, and for Dennis's original contributions on the analysis of distribution, I cannot recommend this as a good book. I looked forward with keen anticipation to reading it, which is perhaps partly why I find it disappointing. But it is the language which really spoils it. For example, on p243 we are told that "Bearing in mind the reticulate control pathways of atmospheric inputs

into local ecosystems, and the original and ultimate dependence of contemporary environments on atmospheric influences, though modified by the interaction of living and non-living components, and thus, in the attempt, to avoid recourse to simplistic determinism, it is suggested that the increasing assumption of oceanic conditions to the north and west of Britain has resulted in a primary structure of selection gradients." Such gems of egregious obfuscation abound throughout the whole work. Only the dedicated will probe below this forbidding mask.

R. I. Vane-Wright

R. I. Vane-Wright is Head of the Butterfly Section at the British Museum (Natural History).

Chemical dynamics

Dynamics of Molecular Collisions. Parts A and B. Modern Theoretical Chemistry. Vol. 1. Pp. 318. \$39.50. Vol. 2. Pp. 380. \$47.40. Edited by W. H. Miller. (Plenum: New York and London, 1977).

IN the past fifteen years, there has been much progress in the field now known as chemical dynamics. The development of the crossed-molecular-beam technique provided a means of studying chemical reactions in more detail, and many new and powerful experimental techniques are now being used in this area. It is now reasonable to ask an ultimate question in kinetics: what is the cross-section for scattering at a particular angle from each state *i* of species *A* to every state *f* of a product *B* at any relative energy? This experimental activity has led chemists into scattering theory; previously, this had been a province of physics. New theoretical techniques and models have been developed by chemists for handling molecular collisions, and some of these are now finding applications in certain areas of physics.

These first two volumes in the series *Modern Theoretical Chemistry* contain thirteen chapters, each by a different author, on theoretical techniques that are appropriate for describing chemical dynamics. The first, by W. A. Lester, Jr, is on the *N* coupled-channel problem. The author likens coupled-channel methods to *ab initio* computations of molecular properties, for they provide a source of reliable data in cases where experiments may be difficult and they serve as a test of approximate methods. The second, by H. Rabitz, is devoted to effective Hamiltonians; it describes various means of reducing the number of rotational

channels that need to be considered explicitly. There is a chapter by D. A. Micha on the use of optical potentials in molecular collisions—their name comes from the analogy between the wave-mechanical aspects of molecular collisions and electromagnetic propagation in an inhomogeneous medium with a complex index of refraction. There is a chapter on vibrational energy transfer by H. K. Shin, and one on the scattering of atoms and molecules from solid surfaces by G. Wolken, Jr. The final chapter in Part A is a very clear account of the theory of non-radiative processes by W. Siebrand.

Part B contains articles on classical and semi-classical methods. It begins with a chapter on classical trajectory methods by R. N. Porter and L. M. Raff. The second chapter, by P. J. Kuntz, is an excellent account of the effect of features of the potential energy surface on molecular collisions. The third chapter, by W. L. Hase, is concerned with the dynamics of unimolecular reactions, and in particular with the RRKM theory. M. S. Child contributes a useful chapter on semi-classical methods and J. C. Tully writes on non-adiabatic processes in molecular collisions. The sixth chapter, by P. Pechukas, is on statistical approximations in collision theory, that is, on the transition-state theory. The final chapter, by R. D. Levine and R. B. Bernstein, is on the thermodynamic approach to collisions; it makes use of information theory.

The topics have been well selected and the authors are known for their work in the field. These two volumes will be very useful to research workers entering the field.

A. D. Buckingham

A. D. Buckingham is Professor of Chemistry at the University of Cambridge, UK.

Mechanisms of animal locomotion

Mechanics and Energetics of Animal Locomotion. Edited by R. McN. Alexander and G. Goldspink. Pp. 346 (Chapman and Hall: London, 1977.) £15.

FOR nearly 30 years from the early 1930s, Sir James Gray and his colleagues at Cambridge published a steady stream of papers analysing how animals move in locomotion. There were distinguished earlier and contemporary contributions by others, but Gray set the foundation for the modern development of the subject. His body of work was largely concerned with the kinetics of locomotion and explained how the movements of most groups of animals exerted appropriate thrusts against the environment and produced locomotion. Some progress was made with explaining the nervous coordination of locomotory movements in a few animals and the energetics of locomotion, but there was little advance in the formidably difficult subjects of the aerodynamics or hydrodynamics of animal movement through fluids. During this period, studies of animal locomotion had their firmest links with comparative anatomy, exemplified in the magnificent and detailed functional analysis of the locomotory musculature of arthropods by Sydney Manton (whose book on arthropod locomotion will be published later this year). The physiological dimensions remained relatively underdeveloped alongside this.

The past 20 years have seen a remarkable change in the subject. Previously, animal locomotion was a specialised, rather isolated topic which could be safely ignored by most zoologists. Now, it has become a coherent, mainstream subject drawing together a great variety of scientific knowledge and expertise. As the preface to this volume says with some justice: "it seems a good time to produce a book".

This is an exceptionally good one. It deals with the entire subject of animal locomotion from muscle physiology and nervous coordination to energetics and the kinetics and dynamics of locomotion. Nearly all the topics discussed are difficult for the non-specialist to penetrate, but here they are introduced in an unusually clear, economical and intelligible style. Every concession is made to the reader, and an undergraduate with no previous knowledge of any of these subjects could read this book without much difficulty and with enormous profit. Inevitably, this has led to some simplification and loss of

detail. There is a good chapter on co-ordination of invertebrate locomotion by Delcomyn and another, shorter, chapter on crawling and burrowing in soft-bodied invertebrates by Trueman and Jones. Otherwise, invertebrates (arthropods in particular) get rather brief treatment and the main emphasis is on the locomotion of vertebrates. Viewed as a whole, however, the book provides an excellent account of the current state of our understanding of the mechanisms of animal locomotion.

The book starts with chapters on the design, mechanics and energetics of muscles involved in locomotion, the coordination of invertebrate and vertebrate locomotion, the energy cost, and then concludes with chapters on terrestrial locomotion, crawling and bur-

rowing, swimming, flying and, finally, the locomotion of Protozoa and single cells. There are good bibliographies at the end of each chapter and the book is well illustrated and produced. The editors are to be congratulated on having recruited a very authoritative team of nine authors and, still more, for having achieved a remarkable consistency of style and uniformity of treatment throughout the book. This is not a compendium of separate, contributed articles as so often happens with multi-author volumes; it has rather been welded into a coherent book.

R. B. Clark

R. B. Clark is Professor of Zoology at the University of Newcastle upon Tyne, UK.

Cell division

Mechanisms and Control of Cell Division. Edited by Thomas L. Rost and Ernest M. Gifford. Pp. vii+387 (Dowden, Hutchinson and Ross: Stroudsburg, Pennsylvania; Halsted/Wiley: New York and Chichester, UK, 1977.) \$31.50; £18.75.

BIOLOGY is suffering a proliferation of books, many of which are simply new combinations of material that has already seen printer's ink at least one and often more times. Sometimes this is justified; a comprehensive well-planned collection can usefully summarise and occasionally illuminate a particular field. This expensive little collection, though containing some new material, does neither. The editors describe it as a "compendium of several areas of cell cycle research, especially those dealing with plants. Some . . . reviews . . . new ideas and the results of recent research" for "researchers and advanced students interested in cell behaviour". The precise purpose is not stated. Was it perhaps simply to market another book? Certainly, the title has all the keywords for the big sell.

In the chapter summarising their elegant dissections of the cell cycle in *Allium* meristems, Giménez-Martin *et al.* point out that in the strict sense "cell division" refers only to mitosis and cytokinesis but is often loosely applied to all processes of proliferation including interphase growth. In making their selection, the editors seem to have been confused about which sense to follow. As a result the collection, which contains some good material, is a hotch-potch.

Gurley *et al.* review their work on biochemical events associated with proliferation of an animal cell-line in culture, Douvas and Bonner re-describe the isolation of contractile proteins from liver chromatin. There follow two interesting reviews of plant hormones and cell proliferation, a list of factors affecting plant cell cycles, a catalogue of structural changes in plant and animal nuclei during replication, two reports on plant cell ultrastructure during mitosis and one of several current hypotheses purporting to explain chromosome movement. The penultimate article is part 1 of an anatomy of cell division in euglenoid algae and the ultimate a detailed description of *all* the stages of mitosis and meiosis and their variations in basidiomycotinal fungi. Several contributions have the prose density of a Patrick White novel.

Despite its dynamic title the collection contains much that is purely descriptive. Being diverse, it lacks the connecting thread that makes a cover-to-cover reading easy or inspiring. Being thinly spread, it fails as a definitive reference source.

The book is strongly bound, but paperbacking would have sufficed and might have halved the price. There is a subject but no author index, nor notes on contributors other than the editors. Many of the numerous electron-micrographs are reproduced with a fuzziness that seriously obscures fine detail. The layout is wasteful: a line diagram of six microtubules occupies a page, as do many other diagrams originally published at a quarter of the size.

L. Martin

L. Martin is a member of the Hormone Physiology Department at the Imperial Cancer Research Fund Laboratories, London, UK.

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For those who are seeking a change in their careers we now offer this new service . . .

APPOINTMENTS WANTED

will appear weekly; the advertising charges are £1.50 or \$3.00 per line (approximately seven words). Minimum 3 lines. Box numbers are automatically given without further charge, all correspondence to:

NATURE CLASSIFIED
4 LITTLE ESSEX STREET,
LONDON WC2R 3LF.

Telephone: 01-240 1101

THE OPEN UNIVERSITY

Faculty of Science

ANIMAL TECHNICIAN

£2,689 — £3,087
(under review)

Applications are invited for the post of Animal Technician in the Faculty of Science, tenable from January 1, 1978, or as soon as possible thereafter. The Animal House services the research and training activities of the Biology Discipline and has breeding colonies of rats, mice and marmosets and holding facilities for rabbits, chicks, fish and amphibia.

The successful candidate, male or female, will assist the Senior Animal Technician in the day to day running of the Animal Unit. Candidates should possess AIAT or equivalent qualifications and have at least six years' experience.

The person appointed will be expected to live within 20 miles of the University. We do offer assistance with removal expenses to make this possible. There is a wide variety of corporation and private housing, and we have an Accommodation Officer to advise and help new appointees. The University offers generous holidays and excellent social and sporting facilities.

Further particulars and application forms are available, by postcard request please, from The Personnel Manager (AT 1), The Open University, P.O. Box 75, Walton Hall, Milton Keynes MK7 6AL, or by telephone from Milton Keynes 63868. Closing date for applications: January 18, 1978. 884(A)

UNIVERSITY OF NOTTINGHAM CANCER RESEARCH CAMPAIGN LABORATORIES

A Grade 3 Laboratory Technician is required in the Cancer Research Campaign Laboratories. This post is funded by a research grant and is offered initially for a period of 2 years. The duties will involve assistance mainly with tissue culture and immunological assays in the laboratory. Some assistance with animal research will also be required. Applicants should preferably have a minimum of 3 years experience in this kind of work and possess appropriate qualifications.

Salary in the range of £2,688 to £3,060 per annum.

Applications in writing to the Establishment Officer, University of Nottingham, University Park, Nottingham NG7 2RD giving the names of two referees and quoting Ref. No. CR 2/12/77. 900(A)

appointment of Director (male or female) Brain Metabolism Unit

The Council invites applications for the post of full-time Director at the MRC Brain Metabolism Unit in Edinburgh, following the resignation of Dr Ashcroft to take up the Chair of Psychiatry at Aberdeen. The successful applicant will be a person of high scientific standing (with or without a medical qualification), with an established reputation as an original scientific investigator. The Unit is at present engaged on experimental and clinical research relevant to psychiatry and neurology. The Council wishes the emphasis on the psychiatric interests of the Unit to be maintained. The title and programme of the Unit will be decided by the Council in the light of the research interests of the new Director.

The Unit is mainly housed in purpose-built research accommodation of approximately 4,000 sq ft (nett). A research team of clinical and non-clinical staff is already in post: the size of the Unit will be a matter for discussion between the Director and the Council. The Director will be responsible for maintaining the strong links already existing with the University of Edinburgh.

The salary for a medically-qualified Director, for whom an honorary clinical contract at consultant level will be sought, will be on the MRC clinical scale, equivalent to NHS consultant grade; if an honorary contract is not appropriate, the salary will be within the range for the Council's Special Appointments Grade, which is equivalent to the University professorial range.

MRC

Medical Research Council

Further information may be obtained from Miss R Withnall, 20 Park Crescent, London W1N 4AL. Applications should be submitted to the Secretary of the Council at the same address, not later than 13 February 1978.

879(A)

THE GOVERNMENT OF THE SYRIAN ARAB REPUBLIC ALEPPO UNIVERSITY

Faculty of Medicine

Invites applicants for the following posts as visiting professors for short periods (minimum 6 weeks) vacant 1977/1978 and 1978/1979.

Internal Medicine: M.R.C.P. or American Board in the following:

- Endocrinology
- Immunology
- Liver Diseases
- Infectious Diseases

Surgery: F.R.C.S. or American Board in the following:

- Anatomy
- Pathology
- Surgical Anatomy
- Surgical Pathology

Paediatrics: M.R.C.P. or American Board, in addition to five years experience.

£660 per month

£230 Accommodation allowance per month

Return tickets for the professor.

Please write for full details to:

The Rector
University of Aleppo
Syrian Arab Republic.

865(A)

ST MARY'S HOSPITAL MEDICAL SCHOOL (University of London)

Paddington, London W2 1PG

POSTDOCTORAL

RESEARCH ASSISTANT

FOR DEPARTMENT OF MEDICINE

Applications are invited for a Senior Research Assistant to work on the regulation of renal blood flow in animals. Project entails the development of techniques for the measurement of blood flow to different regions of the kidney and for the collection of renal lymph. The Medical Unit (Director: Professor W. S. Peart) contains other scientists and doctors working on related projects. Some experience in experimental work with animals required. Candidates should hold a doctorate in one of the Biological Sciences. Post is for 3 years in first instance with salary appropriate to qualifications; it is available on or after February 1, 1978.

Further details obtainable from Dr C. S. Wilcox (Tel: 01-262 1280 Ext. 238). Apply in writing to The School Secretary, St Mary's Medical School from whom application forms should first be obtained.

Closing date for applications January 25, 1978. 888(A)

CSIRO AUSTRALIA RESEARCH SCIENTIST

(2 Positions)

DIVISION OF ANIMAL PRODUCTION

ROCKHAMPTON, QUEENSLAND, PROSPECT, N.S.W.

CSIRO has a broad charter for research into primary and secondary industry areas. The Organization has approximately 7,000 employees—2,300 of whom are research and professional scientists—located in Divisions and Sections throughout Australia.

FIELDS: 1. REPRODUCTIVE PHYSIOLOGY

2. ANIMAL BREEDING

GENERAL: The Division has six broadly-based programs of research on tropical animal production, strategic nutrition of livestock, skin and fleece biology, mineral requirements for livestock production, improvement in reproductive efficiency, and animal breeding and genetics, and has laboratories in Rockhampton, Armidale, Sydney and Perth.

1. REFERENCE NUMBER 245/675

FIELD: REPRODUCTIVE PHYSIOLOGY

LOCATION: ROCKHAMPTON, QLD

DUTIES: To undertake research on causes of infertility in cattle in tropical environments. Particular areas of research would include: improvement in heifer fertility, post-partum anoestrus and selection indices correlated with fertility as an aid in breeding. Some research would be in collaboration with other scientists in the Division at Rockhampton as well as in Armidale and Sydney.

QUALIFICATIONS: Ph.D. degree in an appropriate field or equivalent, with research experience in reproductive physiology.

TENURE: Either a fixed term or indefinite appointment may be negotiated—both carry Superannuation benefits.

2 REFERENCE NUMBER 245/676

FIELD: ANIMAL BREEDING

LOCATION: PROSPECT, N.S.W.

DUTIES: The appointee will join a team undertaking research on improving the efficiency of wool production by sheep. The duties will be to design investigations to clarify the extent to which variation in the biology of the skin and the fleece are genetically determined.

QUALIFICATIONS: Ph.D. degree in an appropriate branch of genetics, or equivalent, plus demonstrable research ability. Preference will be given to applicants with a knowledge of sheep breeding.

TENURE: Indefinite with Superannuation.

SALARY FOR BOTH POSITIONS: Research Scientist or Senior Research Scientist: \$A14,292—\$A20,822 p.a. Outstanding applicants may be offered appointment at a higher level.

Applications (in duplicate), stating **FULL** personal and professional details, the names and addresses of at least two professional referees, and quoting reference number 245/675 or 245/676, should reach: The Personnel Officer, Australian Scientific Liaison Office, Canberra House, Maltravers Street, LONDON WC2R 3EH, by 20th January 1978. Applications in U.S.A. and Canada should be sent to: The Counsellor (Scientific), Embassy of Australia, 1601 Massachusetts Avenue, N.W. WASHINGTON D.C. 20036 U.S.A. 915(A.)

ROYAL POSTGRADUATE MEDICAL SCHOOL has a vacancy for a RESEARCH FELLOW

to work on the Comparison of immunological mechanisms of local and metastatic tumour spread and the effects of immunotherapy, tenable as soon as possible until June 1980.
Salary on the appropriate scale starting at £4,403 p.a. plus £450 London Allowance inclusive.
Application forms from Personnel Officer, RPMS, 150 Du Cane Road, London W12 0HS ref. no. 3/272(N). 918(A)

UNIVERSITY COLLEGE DUBLIN

Applications are invited by the Governing Body of the College for the following full-time statutory post:

PROFESSORSHIP OF CROP HUSBANDRY

Prior to application, further information (including application procedure) should be obtained from Mr J. P. MacHale, Secretary and Bursar, University College, Belfield, Dublin 4.

The closing date for receipt of completed applications is **THURSDAY FEBRUARY 2, 1978.** 947(A)

UNIVERSITY OF NEW ENGLAND

Armidale, New South Wales

PROFESSOR OF ANIMAL SCIENCE

The University of New England is seeking to fill the vacancy created by the impending retirement of Professor N. T. M. Yeates as Professor of Livestock Production.

As Professor of Animal Science (the proposed name for the present Department of Livestock Production) and will be appointed its Head for an initial five year period. In that capacity he will also be responsible, in consultation with the departments involved, for co-ordinating all undergraduate animal science teaching in the Faculty of Rural Science.

At present undergraduate teaching activities of the Department of Animal Science involve three courses in Rural Science and five in Economic Studies, all involving applied aspects of animal production. Research and postgraduate programmes and facilities presently encompass the fields of meat science, wool science, applied reproduction, growth and development and animal climatology.

The appointee must have high standing in a field of animal science and will be expected to develop further the Faculty's association with Australian agricultural industries.

Informal enquiries should be directed to the Dean of the Faculty of Rural Science, in the University.

Conditions of Service include assistance with travel and removal expenses and assistance to buy or build a home.

Superannuation will be on the F.S.S.U. pattern but members of the N.S.W. Superannuation Scheme may continue in that scheme if they wish.

Study leave is available and credit may be given for existing entitlements.

Salary: \$A30,786 p.a.

Further particulars relating to this chair from the Association of Commonwealth Universities (Apts.), or Staff Officer, University of New England, as appropriate.

Applications should include full details of qualifications, experience, research interests and publications and the names and addresses of three referees.

Applicants in the United Kingdom, Europe and America should forward their applications to the Secretary General, Association of Commonwealth Universities (Apts.), 36 Gordon Square, London WC1H 0PF, and send a copy to the Staff Officer.

Other applicants should forward their applications to the Staff Officer, University of New England, Armidale, New South Wales, Australia, without delay.

Closing date for applications **February 24, 1978.** 922(A)

KENYATTA UNIVERSITY COLLEGE—KENYA

(A constituent college of the University of Nairobi)

Applications are invited for the following posts:
ASSOCIATE PROFESSOR—CHEMISTRY DEPARTMENT. Candidates must have a Ph.D. in Chemistry and considerable experience of University teaching at least at Senior Lecturer level. Also required is evidence of successful research experience including the supervision of post-graduate students. Applicants should also have had some involvement in the training of secondary school teachers. Preference will be given to those who are qualified in one of the areas of Physical Chemistry.

ASSOCIATE PROFESSOR—PHYSICS DEPARTMENT. Applicants should hold a Ph.D. in Physics and have substantial University lecturing experience. All research specialities will be considered. Appointees will be required to teach graduate and under-graduate students, to plan and direct research, and participate in all academic activities of the College.

Salary scale K£3,600 to K£4,128 p.a. (at present under review). (K£1=£1.36 sterling). The British Government may supplement salaries by £4,332 p.a. (sterling) for married appointees and £3,042 p.a. (sterling) for single appointees (reviewed annually and normally free of all tax) and provide children's education allowances and holiday visit passages. Terms of service include subsidised housing, membership of S.S.S.F. or F.S.S.U. and a non-contributory medical scheme plus family passages. Detailed application (2 copies) including curriculum vitae and naming 3 referees should be sent airmail no later than January 30, 1978 to Registrar, Kenyatta University College, P.O. Box 43844, Nairobi, Kenya. Applicants resident in U.K. should also send one copy to the Inter-University Council, 90/91 Tottenham Court Road, London W1 0DT. Further details are available from either address. 861(A)



Opportunities in Virology

The Wellcome Foundation Ltd. is a major British-owned international pharmaceutical organisation which formulates, develops, manufactures and markets a wide range of human and veterinary medicines and vaccines.

At our Research Laboratories in Beckenham, Kent, we currently have opportunities for

Virologist—Vaccine Development

We seek a graduate microbiologist to carry out development work in the Department of Virology Research & Development.

The task will be to develop laboratory scale experiments into processes suitable for large scale production, and will involve the technologies of cell culture, virus growth and purification, stabilisation and freeze-drying of vaccines, etc. There will be close liaison with Research and Production scientists. The initial task will be collaboration in the

development of novel poliovirus vaccines. This post offers excellent career prospects to the right applicant. Candidates, probably aged 23-35, should have a good academic degree, although a PhD is not essential. The successful candidate will have a sound grounding in basic Virology, an interest in techniques, the ability to work closely with others and a practical frame of mind. Several years of laboratory experience in Virology would be a great advantage. On-the-job training will be provided.

Research Scientist—Veterinary Viruses

We seek a research scientist, with several years of post-doctoral experience in some relevant area of Virology, to work in the Department of Virology Research and Development on viruses causing disease in animals. Topics of current interest include herpes virus infections of small

and large animals and respiratory virus infections of bovines.

The successful candidate will have demonstrated an aptitude for a career in research. A veterinary qualification would be an added advantage.

Senior Technician—Virology Quality Control

to take technical charge of the Cytology Section of the Department of Viral Products Quality Control. Currently the Section comprises a member of the Scientific Staff and eight technicians. The work of the Section includes quality control of cell culture media and sera; quality control of viral vaccine substrates, the supply of a wide range of mammalian cell cultures; cytogenetic mutagenicity testing and cell culture research and development.

In addition to planning and supervising the work of the technical staff, the person appointed will be responsible for the maintenance of laboratory records, laboratory safety, training and general laboratory administration.

The successful candidate, qualified to BSc or at least HNC level, should have a minimum of 1-2 years' experience in cell culture techniques and karyology. Experience in laboratory administration would be an advantage.

The Laboratories are situated in pleasant parkland surroundings, within easy reach of Bromley and Beckenham, about 12 miles from Charing Cross. Conditions of employment are attractive and include four weeks' holiday, company pension fund, sick pay scheme, subsidised canteen and generous assistance with relocation expenses where appropriate.

Please write, indicating the post in which you are interested and giving details of relevant experience and qualification, to R. V. Sutton, Personnel Officer, The Wellcome Research Laboratories, Langley Court, Beckenham, Kent BR3 3BS.



955(A)

DIABETES RESEARCH

Biochemistry graduate with good degree required to join research group working on isotopically determined amino acid turnover (previous experience with amino acid analyses advantageous). Suitable as Ph.D. project. Application with full curriculum vitae to: Dr P. H. Sönksen, St Thomas's Hospital Medical School, London SE1 7EH. 931(A)

CAMBRIDGE UNIVERSITY APPLICABLE MATHEMATICS

The University expects to fill a **Lectureship or Assistant Lectureship in Applicable Mathematics** (e.g. in the mathematics of subjects such as probability, statistics, operational research, control and systems, or non-physical applications). Applications are invited. Further information from the Director of the Statistical Laboratory, 16 Mill Lane, Cambridge CB2 1SB. Telephone 65621, ext. 252.

Closing date: February 18, 1978. 887(A)

UNIVERSITY OF CAMBRIDGE DEPARTMENT OF PATHOLOGY A TECHNICIAN

is required for a 3 year research project into immunological methods of diagnosis. Considerable laboratory experience essential preferably in bacteriology and biochemistry with H.N.C. or equivalent. Salary in region of £2,700 p.a. Applications in writing to The Superintendent, Department of Pathology, Tennis Court Road, Cambridge CB2 1QP. 874(A)

Product and Process Development

...with the accent on results

Over £7,700

The technology of our range of prepared pet-foods has to take account of a demanding and competitive market in which high quality and nutritional standards need to be maintained against a background of controlled manufacturing costs. Our Development Chemists are the vital focus of a continuing joint effort between marketing, commercial and production management to create products which will satisfy consumer demand and develop processes which will improve company performance.

These two related priorities require co-ordination skills of a high order, as well as the ability to direct projects right through from concept to manufacturing stage. The brief includes the evaluation of existing production processes, in addition to the origination of new ones; and the same applies to product development, where constant improvement of existing dry- and intermediate-moisture foods is as important as the

introduction of new products. In all cases, a sound commercial approach will be the key to effective results.

To be considered for this position you should ideally have proved yourself in a comparable commercial environment. Aged 25-35 with a degree in a relevant scientific discipline (preferably chemistry, biochemistry or food technology), you should have at least 3 years' experience in an R & D function - possibly obtained in process and/or product development within the food industry. This position, based at our modern Peterborough factory where we are putting particular emphasis on the development of dry- and intermediate- moisture foods, could lead to career development opportunities both within the R & D function and beyond. The competitive benefits package includes non-contributory pension and life assurance schemes and practical assistance with relocation.



**Pedigree
Petfoods**

A DIVISION OF MARS LIMITED

Please write briefly or ring for more written information and an application form to: Bob Phillips, Pedigree Petfoods, Melton Mowbray, Leicestershire LE13 1BB. Tel: Melton Mowbray 4141.

951(A)

THE UNIVERSITY OF MANITOBA FACULTY OF AGRICULTURE

Applications are invited for the position of
**PROFESSOR AND HEAD OF THE
DEPARTMENT OF PLANT SCIENCE**

Preference will be given to applicants with teaching experience, holding a Ph.D. degree or equivalent. Proficiency in oral and written communication is essential. A demonstrated competence in a field of plant research related to Canadian agriculture will be an important consideration.

The appointee will be responsible for the administration and co-ordination of teaching and research within the Department, and for liaison with the farm community, government, and industrial agencies.

The Department consists of 21 academic staff members who are active in teaching and research in the areas of agronomy, crop protection, cytogenetics and plant breeding, horticulture, and physiology-biochemistry.

EFFECTIVE DATE OF APPOINTMENT July 1, 1978.

CLOSING DATE FOR APPLICATIONS February 15, 1978.

Salary will be commensurate with experience and qualifications.

Applications, including curriculum vitae, and the names of three referees, should be addressed to:

Dean L. H. Shebeski
Faculty of Agriculture
The University of Manitoba
Winnipeg, Manitoba
R3T 2N2

893(A)

UNIVERSITY OF SYDNEY CHAIR OF PHYSICS (Theoretical Physics)

Applications are invited for the Chair of Physics (Theoretical Physics) which has become vacant on the resignation of Professor S. T. Butler, F.R.S. The School of Physics is concerned with the fields of optical astronomy, radio astronomy, plasma physics, theoretical physics, cosmic ray physics and solid state physics applied to solar energy. Applicants should have qualifications and interests in the general field of theoretical physics and particularly in the fields of plasma physics or astrophysics.

Salary \$A30,786 p.a.

A statement of Conditions of Appointment and Information for Candidates may be obtained from the Registrar, University of Sydney, NSW 2006, Australia, with whom applications close on March 10, 1978. Information also available from Association of Commonwealth Universities (Appts), 36 Gordon Square, London WC1H 0PF. 938(A)

UNIVERSITY OF WISCONSIN Assistant Professor in Biochemistry

The Department of Biochemistry invites applications for the above position. Preference will be given to candidates in the areas of immunobiochemistry, plant biochemistry and X-ray crystallography. Female and minority candidates are especially encouraged to apply. Send detailed curriculum vitae, names of three references and a short statement of research interests to: H. F. DeLuca, Department of Biochemistry, University of Wisconsin, Madison, WI 53706. The University of Wisconsin is an equal opportunity/affirmative action employer. 827(A)

Aslib Director General

- THE Council of Aslib is to appoint a successor to Mr. Leslie Wilson who retires in 1978.
- As chief executive officer the Director General is accountable to the Council for the conduct and development of all aspects of the Association's business, which is to promote the effective communication, management and use of information in industry and commerce, central and local government, education and the professions.
- FORMULATION and interpretation of policy, and relations with member organisations, with industry, commerce, government and the academic world in the UK and overseas are the prime responsibilities.
- THE appointment demands the personal stature that stems from a record of creative achievement over a wide range of managerial responsibility requiring, especially, business acumen and communication skills.
- SALARY is negotiable well into five figures.

Write in complete confidence
to Sir Harold Atcherley as adviser to the Association.

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10 HALLAM STREET and LONDON WIN 6DJ
12 CHARLOTTE SQUARE • EDINBURGH EH2 4DN

869(A)

UNIVERSITY OF OTAGO Dunedin, New Zealand CHAIR OF BOTANY

The University Council invites applications for the Chair of Botany at present occupied by Professor G. T. S. Baylis who is retiring at the end of 1978. Biological sciences are strongly represented in the University, and some preference may be shown towards candidates whose interests would encourage inter-disciplinary co-operation in teaching and research.

Professorial salaries, which are regularly reviewed, are fixed at various points within the range of NZ\$20,804 per annum to \$26,481 per annum, inclusive of cost-of-living allowances.

Further particulars are available from the Secretary General, Association of Commonwealth Universities (Appts), 36, Gordon Square, London WC1H 0PF, or from the Registrar of the University.

Applications close on **February 28, 1978.**
921(A)

UNIVERSITY OF BRISTOL Department of Pharmacology POSTDOCTORAL RESEARCH ASSISTANT Re-advertisement

Applications are invited for a post funded by the Medical Research Council to work on the effects of halothane on neuronal microtubules in collaboration with Dr A. Livingston. Experience in electron microscopy, column chromatography and polyacrylamide gel electrophoresis an advantage.

The appointment will be for two years from March 1978 and the commencing salary will be up to £3,547 per annum and is superannuable.

Applications with the names of three referees should be sent by January 14, 1978 to Dr A. Livingston, Department of Pharmacology, The Medical School, University Walk, Bristol BS8 1TD. 824(A)

INTERIM DEAN FOR VETERINARY PROGRAMS

Tufts University seeks an experienced academic leader for the planning and development of a proposed School of Veterinary Medicine. The School is conceived as an integral part of an academic health center and as a medical, agricultural, and scientific resource for New England. It will join the Tufts University Schools of Medicine and Dental Medicine, the New England Medical Center Hospital, and a major nutrition research center at the Tufts Boston campus, and will share and strengthen existing basic science departments. The School will have its own clinical campus and will also provide clinical education at a number of eminent associated institutions throughout New England. Specialties are expected to develop in comparative medicine, laboratory animal medicine, nutrition, public health, toxicology, pathology, and aquatic animal and equine medicine. Candidates for the Interim Deanship should be veterinarians who have extensive and successful experience in the planning, administration, and support of veterinary institutions or health sciences programs. Distinguished research accomplishments and teaching and clinical experience are also important. Position available immediately. Send nominations and applications to: President Jean Mayer, TUFTS UNIVERSITY, Medford, MA 02155. An Equal Opportunity/Affirmative Action Employer. 940(A)

TECHNICIAN— Electrophysiological Experience

Position available immediately for someone with micro-electrode experience to work with tissue cultured neurons. Electronics and computer experience highly desirable. Tissue culture experience desirable but not essential. Salary to \$10,000 with annual raises. Contact Dr Frederick Sachs, Dept. of Pharmacology, 127 Farber Hall, State University of NY at Buffalo, Buffalo, NY 14214 U.S.A. An Affirmative Action/Equal Opportunity Employer. 833(A)

M.R.C. CLINICAL RESEARCH CENTRE (Northwick Park Hospital) Watford Road, Harrow Middx. HA1 3UJ

The division of communicable diseases is seeking a biochemist with at least 5 years postgraduate virological experience or a virologist of similar experience with biochemical expertise to work on the identification and analysis of the antigens of coronaviruses and the development of methods for detecting them and the corresponding antibodies. Candidates must be over 27 years of age and have a minimum of three years postdoctoral experience.

Salary from £4,607 p.a. to £5,219 plus £450 London allowance. Further details and application form obtainable from Mrs. J. Tucker-Bull. Closing date January 14. Please quote reference 108/1/4/4053. 913(A)

LOTHIAN HEALTH BOARD BASIC GRADE CYTOGENETICIST

There is a vacancy for either a full time graduate or two part time graduates in the Area diagnostic cytogenetics service based at the Pathology Dept. Royal Hospital for Sick Children, Edinburgh. Experience in human cytogenetics desirable. Further particulars of the post can be obtained from Dr A. D. Bain, Pathology Department, Royal Hospital for Sick Children, Edinburgh. Tel: 031-667 1991, Ext. 270.

Salary scale £2,277 per annum to £3,933 per annum plus salary supplement of £312 per annum plus phase II salary supplement with placement according to qualifications and experience.

Applications, which should be type-written, giving particulars of age, qualifications, and previous experience, together with the names and addresses of two referees should be lodged with the Secretary, 11 Drumsheugh Gardens, Edinburgh EH3 7QQ, by 19 January, 1978. 930(A)



Wellcome

Section Head

Chemical Development Laboratories

Natural Products Chemistry

The Wellcome Foundation Limited, a leading British-owned international pharmaceutical organisation, formulates, develops and manufactures a wide range of chemical and pharmaceutical products. Annual turnover is in excess of £290 million with profits being donated to further medical and veterinary research.

We currently have an opportunity for a Section Head, Natural Products Chemistry, within the Process Development Group of the Chemical Development Laboratories at Dartford, Kent.

The post will involve working in close liaison with both research and production teams involved in the development of production scale procedures from research prototypes and the evaluation of new and existing processes, as well as providing a service for the resolution of production difficulties.

Candidates should be organic chemists with a good honours degree or a Ph.D. in Chemistry and several years experience of the specialised methods and techniques required for the purification and isolation of naturally occurring substances (both chemical and proteinaceous) of pharmaceutical interest.

We offer an attractive salary, dependent upon qualifications and experience. Excellent conditions include 4 weeks' annual holiday and generous assistance with relocation expenses where appropriate.

For an application form, please write to or ring
Ian Griffiths, Personnel Division, The Wellcome
Foundation Limited, Temple Hill, Dartford, Kent
Telephone: Dartford 23488, ext. 2061



876(A)

FACULTY OPENING IN BIOCHEMISTRY University of Illinois Champaign-Urbana

The Department of Biochemistry is seeking applicants for a tenure track appointment at the level of Assistant Professor or higher to begin August, 1978. Applicants should be qualified to teach biochemistry courses at undergraduate or graduate levels. Only candidates who have demonstrated excellence in research and who are prepared to establish an active research program will be considered; preference will be given to those who have received graduate and postdoctoral training in strong research environments. The minimum salary at the Assistant Professor level is \$15,000 for a nine-month appointment; salaries at higher ranks are negotiable. For full consideration, application materials, including a curriculum vitae, a list of publications, a brief description of research interests and three letters of reference should be sent by February 1, 1978 to:

Professor Lowell P. Hager, Head
Department of Biochemistry
University of Illinois
Urbana, Illinois 61801
Telephone: (277) 333-3945.

The University of Illinois is an Affirmative Action/Equal Opportunity Employer. 836(A)

DEPARTMENT OF NUTRITION AND FOOD SCIENCE MASSACHUSETTS INSTITUTE OF TECHNOLOGY FACULTY POSITION

The Department, a large multidisciplinary teaching and research unit with activities in all principal areas of nutrition and food science is seeking an applicant to fill the following faculty position:

An M.D., with research training and preferably with a Ph.D. degree, and experience and a major interest in human and clinical nutrition. The appointee will be expected to teach graduate level courses concerned with human and clinical nutrition, to develop a vigorous research programme and become actively involved in the research programmes of the M.I.T. Clinical Research Center.

The appointment will be at the Assistant or Associate Professor rank, depending upon the qualification of the successful applicant.

Curriculum vitae, together with representative publications and the names of five referees should be submitted before February 1, 1978 to: Dr V. R. Young, Chairman Search Committee, Room 56-331, Massachusetts Institute of Technology, Cambridge, MA 02139, USA.

M.I.T. is an Equal Opportunity Employer. Representatives of minority groups are urged to apply. 830(A)

THE OPEN UNIVERSITY



Faculty of Science

RESEARCH ASSISTANT IN BIOPHYSICS

The Research Assistant will work at the Open University Research Unit at Boars Hill, Oxford, on a research project supervised by Professor G. F. Elliott and concerned with biological polyelectrolytes, notably muscle and cornea. Candidates should hold at least a good first degree in Physics, Chemistry or Physiology. The Assistantship is available from January 1978 (or as soon as possible thereafter) to December 1980. Commencing salary will be within the Research Assistant 1B scale £2,904 to £3,333 (age 26) to £4,190 p.a.

Details and application forms are available, by postcard request please, from The Personnel Manager (RAB3), The Open University, P.O. Box 75, Walton Hall, Milton Keynes MK7 6AL, or by telephone from Milton Keynes 63868. Closing date for applications: January 19, 1978.

878(A)

KENYATTA UNIVERSITY COLLEGE—KENYA

Applications are invited for the following posts in the DEPARTMENT OF MATHEMATICS:

SENIOR LECTURER. Applicants should be holders of a Ph.D. or equivalent and must have substantial experience in teaching and research at University level. Ability and interest to plant and execute new course programmes for both undergraduates and postgraduates are essential qualifications. Experience in teaching in a teacher-education institution will be an added advantage.

LECTURER. Applicants should have a good postgraduate degree, preferably a doctorate, in pure or applied mathematics and/or statistics. They should have teaching and research experience at University level.

Salary scales (under review): Senior Lecturer £K2,772 to £K3,600 p.a. Lecturer £K1,800 to £K3,096 p.a. (£K=£1.34 sterling). The British Government may supplement in range £3,354 to £4,128 p.a. (sterling) for married appointees and £2,184 to £2,880 p.a. (sterling) for single appointees (reviewed annually and normally free of all tax) and provide children's education allowances and holiday visit passages. Terms of service include subsidised housing, membership of S.S.S.F. or F.S.S.U. and a non-contributory medical scheme and family passages. Detailed applications (2 copies) including curriculum vitae and naming 3 referees to be sent airmail to Registrar, Kenyatta University College, P.O. Box 43844, Nairobi, Kenya by January 25, 1978. Applicants resident in U.K. should also send one copy to Inter-University Council, 90/91 Tottenham Court Road, London W1P 0DT. Further particulars are available from either address. 860(A)

THAMES POLYTECHNIC SCHOOL OF MATERIALS SCIENCE AND PHYSICS X-RAY AND NUCLEAR LABORATORY TECHNICIAN (Grade 5)

Applications are invited for a technician for the X-ray and nuclear laboratories who will be required to operate X-ray equipment, handle radioactive sources and be responsible for keeping exposure records. Candidates should have a recognised apprenticeship or H.N.D./H.N.C. qualification and at least eight years' relevant experience.

Salary scale: £3,027 to £3,483 including London weighting plus personal allowance (i.e. 5% of gross pay; min. £2.50, max. £4.00).

Further particulars and form of application may be obtained from the Staffing Officer, Thames Polytechnic, Wellington Street, London SE18 6PF, to whom completed applications should be returned as soon as possible. 903(A)

UNIVERSITY OF NAIROBI KENYA

Applications are invited for the following posts in the

DEPARTMENT OF AGRICULTURAL ECONOMICS

1. ASSOCIATE PROFESSOR IN PRODUCTION ECONOMICS AND FARM MANAGEMENT

Applicants must possess a good honours degree in Agricultural Economics, Economics or Agriculture and a Ph.D. in Agricultural Economics. Sound knowledge of planning techniques and decision theory is essential, supported by research experience and reviewed publications. Academic duties will mainly concern lecturing and research supervision of M.Sc. and Ph.D. students in the fields specified. Occasional support lecturing in general economics and research methods subjects will be expected. Administration responsibilities will involve those associated with a senior appointment in an active and growing Department.

2. SENIOR LECTURER

Applicants, who should possess a Ph.D. must have relevant teaching and research experience in Agricultural Economics at University level. The appointee should have a good background in Economic Theory and Quantitative Techniques and should be capable to apply these in the field of agriculture. The appointee will be expected to teach at both undergraduate and graduate levels. Experience in supervision of research is essential.

Salary Scales: Associate Professor K£3,600 to K£4,128 p.a. Senior Lecturer K£2,772 to K£3,600 p.a. (K£1=£1.35 sterling). Salaries are currently under review. The British Government may supplement salary in range £4,128 to £4,332 p.a. (sterling) for married appointee or £2,880 to £3,042 p.a. (sterling) for single appointee (normally free of all tax and reviewed annually) and provide children's education allowances and holiday visit passages. F.S.S.U.; family passages; various allowances. Detailed applications (2 copies) including curriculum vitae and naming 3 referees should be sent air mail to Registrar, (Recruitment and Training), University of Nairobi, P.O. Box 30197, Nairobi, Kenya. Closing date January 27, 1978. Applicants resident in U.K. should also send 1 copy to Inter-University Council, 90/91 Tottenham Court Road, London W1P 0DT. Further details may be obtained from either address. 894(A)

UNIVERSITY OF SUSSEX BIOCHEMISTRY LABORATORY POSTDOCTORAL RESEARCH FELLOW

Applications are invited for a position sponsored by the Wellcome Trust to join a group studying the control of proliferation in mitogen-stimulated lymphocytes. Candidates should have a background in biochemistry or cell biology.

Salary will be on the Research Fellow 1A scale, £3,333-£5,627 p.a., and the post is available until September 30, 1979. Applications, with curriculum vitae and the names of two referees, should be sent to Dr John Kay, School of Biological Sciences, University of Sussex, Falmer, Brighton BN1 9QG. 944(A)

MEDICAL RESEARCH COUNCIL NATIONAL INSTITUTE FOR MEDICAL RESEARCH

W.H.O. Special Programme for Research and Training in Tropical Diseases

A vacancy exists for a cell biologist or biochemist to join a group working on the control of variable antigen synthesis in malaria parasites. The appointment will be for up to three years and will be in the present salary range £3,995 to £5,219 p.a. according to age and qualifications plus £450 p.a. London Allowance; superannuation provision. Recent Ph.D. graduates or students expecting to complete their doctorate in 1978 are invited to apply before February 12, 1978.

Applications, giving details of qualifications, experience and the names of two professional referees, should be sent to the Director, National Institute for Medical Research, Mill Hill, London NW7 1AA. 886(A)

Electron Microscopist

Applications are invited from experienced microscopists (preferably graduates in a Biological Science) to take full responsibility for a comprehensive Services Section within the Research Division of Beecham Pharmaceuticals.

The section provides a service to the Division encompassing all aspects of the R & D programmes, including many microbiological and biomedical problems. This post will present an opportunity to expand the application of a wide range of techniques in a multi-disciplinary industrial research environment.

The section is well equipped with a Philips EM 300 TEM, a new Philips SEM 501 and Zeiss microscope facilities. There is provision for histology, ultramicrotomy, freeze etch/vacuum coating and other routine preparatory systems besides comprehensive dark room equipment, within a purpose-built suite.

Ideally, applicants, male or female, should have experience in electron microscopy applied to histopathology and/or microbiology, with a broad technical knowledge of light microscopy, transmission and scanning E.M. and associated techniques. A practical and innovative approach is essential.

Brockham Park is situated near Dorking, free transport being available to and from local stations. Assistance can be given in finding accommodation in the area. The Company will offer a good starting salary and benefits which include a non-contributory pension scheme, free life assurance, flexible working hours, excellent sports and social facilities, etc.

Please apply, summarising professional qualifications and experience to:

Miss E A Gunn, Site Personnel Officer, Beecham Pharmaceuticals Research Division, Brockham Park, Betchworth, Surrey RH3 7AJ

Beecham

Pharmaceuticals

880(A)



UNIVERSITY OF DUBLIN

Trinity College

CHAIR OF CHEMISTRY

and

HEADSHIP OF DEPARTMENT

Applications are invited for appointment to these posts which will fall vacant on October 1, 1978, following the retirement of the present holder Dr Wesley Cocker. While the University is not committed to an appointment solely in one field of Chemistry, applications from persons with a special interest in Inorganic Chemistry will be particularly welcome.

Further particulars, both of the present activities of the department of Chemistry, and of the conditions of appointment to the Chair and to the Headship, may be obtained from:

Mr G. H. H. Giltrap, Secretary to the College,
Trinity College, Dublin 2

to whom formal application should be made, preferably not later than January 23, 1978.

571(A)

FORENSIC SCIENCE

Opportunities for Chemists/Biochemists

Opportunities in Forensic Science

Forensic investigation makes wide-ranging demands on the abilities of chemists and biochemists. Serology, enzymology and immunology techniques are employed in comparing blood, saliva and semen stains. Identification of plant and animal materials and textile fibres demands a variety of chemical and taxonomic approaches, and skilled use of the microscope. The modern chemical and physical methods of analysis are involved in the investigation of paint, glass and trace materials, whilst toxicology and drug identification require some specialised knowledge of organic analysis.

WHERE YOU COULD WORK

Vacancies exist at Cardiff and Bristol (both moving to Chepstow in 2-3 years), and at Aldermaston, Birmingham, Chorley and Wetherby.

The successful candidates will initially help experienced colleagues. Later, they will take responsibility for their own investigations and reports, and act as expert witnesses. Their work may at first overlap several of the areas mentioned above, but they will have opportunities to specialise.

Candidates, normally aged under 27, must have a degree, or equivalent qualification, in Biochemistry or Chemistry. Those with experience in any of the above fields are particularly invited to apply. *Final year students will not be considered.*

Appointment will be as Scientific Officer, with starting salary within the range of £2590-£4030. There are promotion prospects and a non-contributory pension scheme.

For further details and an application form (to be returned by 20 January 1978) write to Civil Service Commission, Alencon Link, Basingstoke, Hants. RG21 1JB, or telephone Basingstoke (0256) 68551 (answering service operates outside office hours). *Please quote Ref. SB/17/LA.*

HOME OFFICE 939(A)



GLASGOW INSTITUTE OF RADIOTHERAPEUTICS AND ONCOLOGY

Applications are invited for the position of
SENIOR SCIENTIFIC OFFICER

The successful candidate will lead a small team who will be investigating the biological effectiveness of various radiotherapy regimes. This is a postdoctoral appointment supported by an M.R.C. Project Grant. Candidates need not necessarily be radiobiologists but they should have worked with small animals and some knowledge of biophysics would also be useful. The appointment is for three years from April 1, 1978. The salary is that for the Senior Scientist grade in the National Health Service in the range £4,957 to £5,362.

Applications with curriculum vitae, two referees and a day-time telephone number should be sent before January 27 to:-

Dr A. H. W. Nias,
Radiotherapy Department,
Belvidere Hospital,
Glasgow G31 4PG.
(Tel. 041-554 1855)

946(A)

ASSISTANT/ASSOCIATE PROFESSOR DEPARTMENT OF BIostatISTICS

Ph.D. in Statistics, Applied Mathematics or equivalent background, plus teaching and consulting experience in applications of statistics or mathematical methods to problems in biology, medicine, or public health. Applications including CV and three letters of recommendation to Dr Frederick Mosteller, Department of Biostatistics, Harvard School of Public Health, 677 Huntington Avenue, Room 706, Boston, MA 02115. Harvard University is an Equal Opportunity/Affirmative Action Employer. 912(A)

GUY'S HOSPITAL MEDICAL AND DENTAL SCHOOLS RESEARCH ASSISTANT (Immunologist/Biochemist)

Pre- or postdoctoral immunologist or biochemist having experience in immunochemical techniques required for a period of two years in the first instance. The person is required to investigate immune responses to Streptococcus mutans in rhesus monkeys.

Salary according to qualifications and experience, with £450 per annum London Allowance and superannuation.

Apply in writing, with curriculum vitae, to the Secretary, Guy's Hospital Medical School, London Bridge SE1 9RT, quoting Ref. I.M.4. 924(A)

DEPARTMENT OF PHYSICS ASSISTANT PROFESSORSHIP

Subject to adequate funding, a position may become available July 1, 1978 in one of the following fields:

QUANTUM OPTICS BIOPHYSICS

Salary depends on qualifications. The closing date for applications is March 31, 1978.

Exceptional candidates in other fields may be considered.

All correspondence should be addressed to:

Professor Luis de Sobrino, Chairman,
Appointments Committee,
Department of Physics,
The University of British Columbia,
2075 Wesbrook Place,
Vancouver, British Columbia,
Canada V6T 1W5. 823(A)

THE UNIVERSITY OF LEEDS RESEARCH FOR SCHOOL SCIENCE MASTERS

Applications are invited from graduate Science Masters in Schools to take part in research in the Procter Department of Food Science for a period of four weeks in the summer vacation. Two places are available in 1978 and grants to cover expenses incurred are available from the Worshipful Company of Skinners through its Lawrence Atwell's Charity. The research will be of a chemical or physical nature and topics will be selected with subsequent continuation in school laboratories in mind either as part of the school science syllabus or as a research project.

Applications, giving details of career and interests, and the names of two referees, should be sent as soon as possible to the Head of Department, Procter Department of Food Science, University of Leeds LS2 9JT. 948(A)

KING'S COLLEGE HOSPITAL MEDICAL SCHOOL (University of London)

Denmark Hill, London SE5 8RX
DEPARTMENT OF

OBSTETRICS & GYNAECOLOGY

Applications are invited from graduates or qualified people for the post of Research Technician on a project mainly involving tissue culture and cytogenetics as well as histological and histochemical techniques in the area of prenatal diagnosis of inherited diseases.

Starting salary £3,112 inclusive.

For informal enquiries please contact Dr J. Singer on 01-274 6222, extension 2349. Applications in writing giving full details of qualifications and experience should be sent to the Secretary of the Medical School at the above address by January 19, 1978. 917(A)

THE UNIVERSITY OF JUBA—SUDAN

Applications are invited for the following posts in the

COLLEGE OF NATURAL RESOURCES AND ENVIRONMENTAL STUDIES

1. PROFESSOR / READER / SENIOR LECTURER / LECTURER IN ANIMAL PRODUCTION.
2. PROFESSOR / READER / SENIOR LECTURER / LECTURER IN FISHERIES.
3. PROFESSOR / READER / SENIOR LECTURER / LECTURER IN WILDLIFE.
4. PROFESSOR / READER / SENIOR LECTURER / LECTURER IN FORESTRY.
5. PROFESSOR / READER / SENIOR LECTURER / LECTURER IN CROP PRODUCTION.
6. PROFESSOR / READER / SENIOR LECTURER / LECTURER IN SOIL CHEMISTRY.
7. PROFESSOR / READER / SENIOR LECTURER / LECTURER IN GENERAL BIOLOGY (Botanist).
8. PROFESSOR / READER / SENIOR LECTURER / LECTURER IN MATHEMATICS.
9. PROFESSOR / READER / SENIOR LECTURER / LECTURER IN PHYSICS.

Salary scales: Professor £54,135 p.a. Reader £53,625 p.a. Senior Lecturer £52,465 to £53,000 p.a. Lecturer £51,600 to £52,400 p.a. (£51=£50.63). The British Government may supplement salaries in range £2,982 to £3,558 p.a. (sterling) for married appointees or £1,410 to £1,980 p.a. (sterling) for single appointees (normally free of all tax and reviewed annually) and provide children's education allowances and holiday visit passages. Family passages; various allowances; superannuation scheme; annual overseas leave. Salary scales and rates of supplementation are under review. Detailed applications (2 copies), including a curriculum vitae and naming referees, should be sent by air mail, not later than January 20, 1978 to the Secretary General, University of Juba, P.O. Box 82, Juba, Sudan. Applicants resident in U.K. should also send 1 copy to Inter-University Council, 90/91 Tottenham Court Road, London W1P 0DT. Further particulars may be obtained from either address. 858(A)

THE QUEEN'S UNIVERSITY
OF BELFAST

DEPARTMENT OF CHEMISTRY

POSTDOCTORAL
RESEARCH FELLOW/
RESEARCH ASSISTANTS

Applications are invited for three positions for work with Dr J. J. Rooney and Professor K. J. Ivin on the mechanism of olefin metathesis, especially in relation to the ring-opening polymerization of cycloalkenes:

1. Research Fellow: £3,333 to £4,403 (under review) with F.S.S.U./U.S.S.
2. Research Assistant: £2,904 to £4,190 (under review) with F.S.S.U./U.S.S.

One research assistant post will be tenable for two years. The other posts will be tenable for one year initially and may be renewed annually for further periods. Commencing salary will be at point appropriate to age, qualifications and experience.

Applications, giving a curriculum vitae and the names and addresses of two referees, should be sent to The Personnel Officer, The Queen's University of Belfast BT7 1NN as soon as possible. 866(A)

LINCOLN COLLEGE

New Zealand

RESEARCH ECONOMIST
(RUMINANT PRODUCTION SYSTEMS)
AGRICULTURAL ECONOMICS
RESEARCH UNIT

Applications are invited for the above mentioned position from persons with higher degree qualifications in Agricultural Economics/Management or Agricultural Science. Applicants should have some experience in the application of quantitative techniques to both production and management problems.

Commencing salary according to qualifications and experience within the range of NZ\$7,386 to \$12,543 per annum. At present salaries are supplemented by an "Interim Special Allowance" of 3.5%.

Travel and removal expenses reimbursed up to specified limits. Government Superannuation available.

Conditions of Appointment are obtainable from the Association of Commonwealth Universities (Apts), 36, Gordon Square, London WC1H 0PF, or from the Registrar of the College.

Applications close on February 17, 1978. 923(A)

NATIONAL COUNCIL
FOR SCIENTIFIC RESEARCH

Applications are invited from suitably qualified persons for the post of:

MATERIALS ENGINEER/
SCIENTIST

Applicants for the post must have a degree in Materials Engineering/Science with a minimum of 5 years experience in research and development work.

The successful candidate will be required to initiate and carry out research and development work on the utilisation of local raw materials in the production of soft boards or development of alternative roofing materials for low cost housing, and to provide technical advice on the production and usage of building materials.

Salary will be according to qualifications and experience on the following scales:

Senior Principal Professional Officer: K7,812 x 204 - K8,016 x 216 - K8,232

Principal Professional Officer: K6,756 x 216 - K6,972 x 288 - K7,200 x 204 - K7,608

Senior Professional Officer: K5,424 x 288 - K5,712 x 300 - K6,012 x 312 - K6,324

Applications giving full personal details, qualifications, experience and names and addresses of three referees should be submitted to:

The Secretary General,
National Council for Scientific Research,
PO Box CH 158,
LUSAKA,
ZAMBIA.

934(A)



Wellcome

Immunologist

Department of Experimental Immunobiology

The Wellcome Foundation Limited is a major British-owned international pharmaceutical organisation. Our Research Laboratories at Beckenham are looking for a post-doctoral scientist to work on a programme concerned with the effect of pharmacological agents on experimental autoimmune disease. Particular emphasis will be directed towards an analysis of the involvement of suppressor cells. The appointment will be available from 1 April 1978, for a period of 2-3 years in the first instance.

Applicants should be able to provide evidence of an innovative approach to immunobiological research.

The starting salary will depend upon experience but is not expected to be less than £4,500 p.a. Excellent working conditions and benefits include generous relocation expenses, where appropriate.

Please apply, giving details of qualifications and previous research experience, to the Personnel Manager, The Wellcome Research Laboratories, Langley Court, Beckenham, Kent BR3 3BS



877(A)

Chemical Defence Establishment, Porton Down, Wilts.

Biologist/Chemist

... to supervise the work of the Tissue Culture Unit in the Medical Division of the Department of Toxicology and Pathology.

The successful candidate will implement and develop suitable tests to use in the assessment of short and long term toxicity in vitro. Cell and tissue culture methods will be applied to a study of the toxicological and biological activity of a wide range of chemicals. The use of rapid screening techniques to complement current toxicological studies will be important. The work involves development and use of in vitro tests for mutagenicity and carcinogenicity.

Applicants, normally aged under 32, must have a good honours degree, or equivalent, in Biology or Chemistry and have post graduate experience in mammalian tissue cell culture techniques. A working knowledge of histology, histochemistry, photomicrography, cinemicrography and autoradiography would be desirable.

Appointment will be as Senior Scientific Officer (£4,700-£6,300) or Higher Scientific Officer (£3,740-£4,975) according to age, qualifications and experience. Starting salary may be above the minimum at each level.

For application form (to be returned by 27 January 1978) write to:
Mr F. C. G. Philbrick, Chemical Defence
Establishment, Porton Down, Wiltshire
Please quote ref: SA/3/FM

881(A)



DIRECTOR

Division of Cancer Cause and Prevention National Cancer Institute

The National Cancer Institute is recruiting for a scientist who will direct, coordinate, and conduct:

- *A nationwide program of laboratory, field, biometric, and epidemiologic research on the cause and natural history of cancer and the means for preventing cancer through an intramural and collaborative program.
- *A program that serves as the focal point for the Federal Government on the synthesis of clinical, epidemiological, and experimental data relating to cancer cause and prevention.
- *A program that evaluates mechanisms of cancer induction by viruses, environmental carcinogenic hazards and the natural history of neoplasms.
- *The research work of a professional and support staff of 590 employees.

Candidates for this position should have an advanced professional degree (Ph.D., M.D., or equivalent) in a medical, biological, physical or related health science, and at least three years of professional specialised experience in Biomedical Science Administration involving two or more Scientific Disciplines (e.g., chemistry, biology, microbiology, etc.). The candidate's professional experience must demonstrate the successful program planning, evaluation, and administration of high caliber biomedical research or clinical programs relating to human disease problems, and extensive experience as an independent investigator in clinical, basic, applied, developmental, or epidemiological research activities. Candidates will be evaluated on their scientific leadership and experience, scientific recognition, training and education, and honors and awards. The filling of this position is subject to the approval of the Civil Service Commission.

This is a career Civil Service position at the Supergrade level with liberal government benefits and salary ranging from \$42,423 to \$47,500. Appointment may also be made through the Commissioned Corps of the U.S. Public Health Service.

Submit Curriculum Vitae, Bibliography and Personal Qualifications Statement (SF-171) by 10 February, 1978, to: Mrs. Elizabeth W. Stroud, NCI Personnel Office, National Institutes of Health, Public Health Service, 9000 Rockville Pike, Building 31, Room 3A19, Bethesda, Md. 20014. An Equal Opportunity Employer M/F.

NATIONAL INSTITUTES OF HEALTH

843(A)

TECHNICIAN

required in Muscle Research Centre, Hammersmith Hospital, to work principally on electron microscopy of muscle. O.N.C./H.N.C. or equivalent and experience in E.M. techniques preferable. Post tenable until October 1978 in the first instance. Salary according to age and experience on the scale £2,982 to £3,808 p.a. plus London Weighting.

Applications with full curriculum vitae and names and addresses of two referees to Professor V. Dubowitz, Department of Paediatrics and Neonatal Medicine, Hammersmith Hospital, Du Cane Road, London W12 0HS. 899(A)

PRINCETON UNIVERSITY DEPARTMENT OF BIOCHEMICAL SCIENCES

Assistant Professor position: Biochemist or molecular or cell biologist with strong background in either physical biochemistry or protein chemistry. Applicants should send vitae, bibliography, list of referees and outline of research program to: Dr Jacques R. Fresco, Chairman, Department of Biochemical Sciences, Princeton University, Princeton, NJ, NS, 08540. An affirmative action/equal opportunity employer.

873(A)

IMPERIAL CANCER RESEARCH FUND Bursaries for Training in Research

A small number of Bursaries will be awarded at the laboratories in Lincoln's Inn Fields for full-time studies leading to higher Degrees in research fields relevant to cancer, that include chemistry, cell and molecular biology.

The awards will be tenable for three years from 1st October, 1978 with a non-superannuable grant of £2,640 a year (subject to tax) and, in some cases, additional allowances.

Applications are invited from British subjects, normally resident in the U.K. and not over 25 years of age, who hope to obtain First or Upper Second class Honours Degrees in Science in 1978.

Further details and application forms are available from:

The Personnel Officer
Imperial Cancer Research Fund
Lincoln's Inn Fields
London WC2A 3PX

Completed applications should reach the Director of Research at the above address not later than 14th February, 1978. 904(A)

UNIVERSITY OF READING LECTURESHIP IN PHYSIOLOGY & BIOCHEMISTRY

Applications are invited for the above post from graduates in Biochemistry, Physiology, Pharmacology or Veterinary Science, with active research interests in some area of pharmacology. The person appointed should take up duties as soon as possible.

Further information may be obtained from the Registrar (Room 214, Whiteknights House), The University, Whiteknights, Reading RG6 2AH, by whom applications should be received not later than February 1, 1978. 837(A)

UNIVERSITY OF SOUTHAMPTON DEPARTMENT OF CHEMISTRY

POSTDOCTORAL RESEARCH FELLOW, salary range £3,333—£3,547. One year appointment to start as soon as possible. The project concerns the study of absorption at metal electrodes by modulated specular reflectance spectroscopy. Experience in electrochemistry or the study of surfaces by optical methods will be a considerable advantage. A curriculum vitae and the names of two referees should be sent to Dr A. Bewick, Department of Chemistry, The University of Southampton, Southampton SO9 5NH. 936(A)

THE UNIVERSITY OF SHEFFIELD DEPARTMENT OF BUILDING SCIENCE

Applications are invited from men and women for 2 POSTDOCTORAL RESEARCH POSTS, RANGE 1A, and 1 RESEARCH ASSISTANT RANGE 1B.

Post A: Range 1A up to £3,761 a year.

Tenable for three years. To work on the environmental performance of windows with special reference to energy conservation, solar energy and climatic design using interactive computing techniques. Postgraduate research experience in building environmental engineering, building climatology, physics or some other related discipline, plus some experience in computer programming in FORTRAN required.

Post B: Range 1B up to £3,761 a year.

Tenable for three years. To work on the assessment of dynamic wind loads on buildings using small scale aerodynamic models in wind tunnels. Postgraduate research experience in wind engineering research required. Knowledge of structural dynamics and/or mini computer analysis would be useful.

Post C: Range 1B up to £3,119 a year.

Tenable for 1 to 2 years. To work on geographical variations in the transmission of the atmosphere in the U.K. for solar radiation in relation to other climatological factors influencing heat transfer from buildings. Suitable graduate experience in Applied Climatology, Applied Meteorology or Environmental Science required. Experience in the use of digital computers would be an advantage.

Further particulars from the Registrar and Secretary, The University, Sheffield S10 2TN to whom applications should be sent by January 20, 1978. Quote Ref. R. 62/G. 949(A)

AUSTRALIAN WINE RESEARCH INSTITUTE Adelaide, South Australia RESEARCH MICROBIOLOGIST

Duties: To carry out a programme of microbiological and biochemical research in oenology; this would include yeasts and lactic acid bacteria with studies of related enzymatic processes in winemaking; to investigate and advise on problems of spoilage of wine, and to maintain the Institute's yeast culture collection.

Qualification and Experience: Ph.D. or equivalent research experience in relevant field. Some background in biochemistry and microbiology. Skill in sensory evaluation would be an advantage.

Salary: Research Scientist \$14,292 to \$17,548 p.a., plus immediate entry to a contributory superannuation scheme.

Applications, which will be treated in strictest confidence, should include full personal and professional details and the names of at least two referees and should be submitted to:-

The Chairman,
Council of the Australian
Wine Research Institute,
Post Office,

Glen Osmond, South Australia, 5064.
by January 31, 1978. 909(A)

THE UNIVERSITY OF THE WEST INDIES, TRINIDAD

Applications are invited for the vacant temporary post of SENIOR LECTURER/TURER/ASSISTANT LECTURER in the DEPARTMENT OF LIVESTOCK SCIENCE, FACULTY OF AGRICULTURE. Preference will be given to applicants holding a first degree in animal science or veterinary medicine with postgraduate qualifications in animal physiology. Interest and experience in the physiology of dairy cattle reproduction (infertility) and/or adaptation of ruminants to hot, humid climates would be an advantage. The appointment will be for 2 years with the possibility of extension for a total of 5 years. Salary Scales (1977-78): Senior Lecturer: TT\$25,827 to TT\$34,017 p.a. Lecturer: TT\$19,071 to TT\$29,799 p.a. Assistant Lecturer: TT\$15,480 to TT\$16,974 p.a. (£1 sterling = TT\$4.39). F.S.S.U. Unfurnished accommodation if available at 10% or furnished at 12½% or housing allowance of 20% of pensionable salary. Up to five full economy passages on appointment and on normal termination. Study and Travel Grant. Detailed application naming three (3) referees to Secretary, U.W.I., St Augustine, Trinidad. Details of post sent to all applicants. 859(A)

RHODES UNIVERSITY

Grahamstown, South Africa

**Applications are invited for the post of
SENIOR LECTURER/LECTURER
IN ZOOLOGY**

from July 1, 1978 or as soon as possible thereafter.

The salary scales are:-

SENIOR LECTURER: R8,460 by R360 to R9,900 by R450 to R11,250 per annum.**LECTURER:** R6,300 by R360 to R9,180 per annum.

(Note: £1 sterling=approximately R1.59)

The initial salary will be determined according to qualifications and experience. In addition a supplement of 10% on the above scales and a vacation savings bonus are payable. The successful applicant will become a member of the University's pension and medical aid schemes.

The successful applicant will be required to teach invertebrate biology. Research interests in either freshwater or estuarine biology will be a strong recommendation.

Further particulars and application forms may be obtained from the Registrar, Rhodes University, Grahamstown, 6140, South Africa to whom completed applications together with a recent photograph should be sent by March 15, 1978. 895(A)

**UNIVERSITY OF SURREY
DEPARTMENT OF BIOCHEMISTRY
RESEARCH OFFICER**

An interesting vacancy exists for a Research Officer to work on Tryptophan and its metabolites with particular reference to biological rhythms.

The vacancy is for two years and would suit a graduate (male or female, under 25 years of age) in a biological subject, preferably with some experience of fluorimetry and/or radio immunoassay and an interest in the biochemistry of the nervous system.

The salary is £2,904 p.a., but this is at present subject to review.

Applications in the form of a curriculum vitae, including the names and addresses of two referees, should be sent to the Assistant Secretary (Personnel), University of Surrey, Guildford, Surrey GU2 5XH, as soon as possible. 916(A)

**UNIVERSITY OF BRISTOL
DEPARTMENT OF PHYSICS
RESEARCH ASSISTANT**

Applications are invited for a Research Assistantship funded by N.E.R.C. for an investigation of the frost cracking of rocks. The study would involve laboratory and theoretical work to elucidate the basic physics involved when ice freezes in a crack in a solid, generating stresses which propagate the crack. Photoelastic techniques would be used; some research experience in crack problems is desirable. The appointment is for three years with a starting salary of £3,761 p.a. (under review).

Applications, with curriculum vitae, details of research experience and names and addresses of two referees, should be sent to Professor J. F. Nye, H. H. Willis Physics Laboratory, Tyndall Avenue, Bristol BS8 1TL by February 2, 1978. 871(A)

**THE UNIVERSITY OF LEEDS
THE UNIT FOR CANCER RESEARCH**

Applications are invited for the post of BIOCHEMIST to join a research team in the above Unit and the Department of Chemical Pathology. Candidates should have previous clinical laboratory experience. The team is studying biochemical discriminants in cancer treatment, the work will involve development of protein and enzyme analysis and their evaluation in cancer patients.

Salary on the IB Scale for Research and Analogous Staff: £2,904 to £4,190. Appointment will be for one year with the possibility of renewal for a further two years.

Apply to: Professor E. H. Cooper, Unit for Cancer Research, University of Leeds, Leeds LS2 9JT. 907(A)

Scientist for Research Product Management

In the Boots Company the activities involving the development of new medical and over the counter pharmaceutical products are co-ordinated and progressed by Research Product Managers (Pharmaceutical). We wish to appoint a graduate to assist the Senior Research Product Manager (Pharmaceutical) in these duties which require liaison with technical staff in Research and many other departments including Marketing, Quality Control and Production and the use of modern control techniques.

If you have a formal qualification, preferably in pharmacy, pharmacology or chemistry, some relevant experience in the pharmaceutical industry, the ability to co-operate with staff of various disciplines and to communicate effectively - then, you are the candidate we seek.

Conditions of employment include contributory pension and profit earning bonus schemes. Help with relocation is available if appropriate.

Please write for application form to: T. W. Flower, Employment Manager (Technical),

The Boots Company Ltd.,
Station Street, Nottingham NG2 3AA.

896(A)

**ASSISTANT PROFESSOR
OF APPLIED MATHEMATICS
DEPARTMENTS OF PHYSIOLOGY AND
BIOSTATISTICS**

Responsibilities include teaching of statistics and the conduct of collaborative research in pulmonary physiology. Essential are competence in mathematical modelling and in experimental design and analysis. Familiarity with pulmonary biology and computing is desirable. Address inquiries to Dr Joseph Brain, Department of Physiology, Harvard School of Public Health, 665 Huntington Avenue, Boston, MA 02115. Harvard University is an Equal Opportunity/Affirmative Action Employer. 911(A)

**NATIONAL INSTITUTE FOR
RESEARCH IN DAIRYING
(University of Reading)
FEEDING AND METABOLISM
DEPARTMENT**

Applications are invited for a scientist to undertake routine supervision and control of nutritional experiments on lactating cattle and young stock. The person appointed will also be expected to contribute to the planning and interpretation of the trials, including some statistical analysis.

Pass degree, H.N.C. in animal production or animal nutrition with physiology or equivalent experience is required. Practical dairy farming experience, current driving licence, and some knowledge of statistics would be advantageous.

Appointment will be as Scientific Officer (£2,592 to £4,032) or Higher Scientific Officer (£3,745 to £4,976) including current pay supplements, according to experience. At least five years' relevant postgraduate research or other approved experience is required for appointment as H.S.O.

Application forms and further details are obtainable from the Secretary, N.I.R.D., Shinfield, Reading RG2 9AT. Quote reference 77/41. 891(A)

**THE MIDDLESEX HOSPITAL
MEDICAL SCHOOL
(University of London)
DEPARTMENT OF
NEUROLOGICAL STUDIES
RESEARCH ASSISTANT**

Applications are invited from graduates for the post of Research Assistant to assist in a variety of laboratory projects connected with research in cerebrovascular disease (strokes). Experience with physiological and/or biochemical techniques an advantage. The post is available for two years.

Salary, according to age and experience, within the range £3,354 to £4,212 per annum (including £450 per annum London Allowance).

Applications, including curriculum vitae and the names and addresses of two referees should be submitted to Dr M. J. G. Harrison, Department of Neurological Studies, Sir Jules Thorn Institute of Clinical Science, The Middlesex Hospital Medical School, London W1. 882(A)

**THE MASSACHUSETTS
GENERAL HOSPITAL**

An opening for a Research Associate is available starting July 1, 1978. M.S. or Ph.D. in Biochemistry or Cell Biology with research interest in any of the following areas: cell membrane changes in differentiation and malignancy; cell surface membrane synthesis, particularly glycoprotein synthesis; in vitro protein synthesis in mammalian systems; calcium absorption and Vitamin D metabolism; Vitamin A metabolism.

The Research Associate will be part of a new program in Gastrointestinal and Nutritional Research being established at the State University of New York in Buffalo. An opportunity is also available for qualified applicants for an associated academic appointment in the Department of Biochemistry. Please send résumé to: Dr Milton M. Weiser, Chief of G.I. and Nutrition, S.U.N.Y., at Buffalo, c/o G.I. Laboratories, Massachusetts General Hospital, Boston, MA. 02114. 928(A)

Research Associateships are available for

Theoretical Nuclear Physicists

to join the Theory Group at Daresbury whose fields of interest are nuclear, solid state and atomic and molecular processes.

At the Laboratory a major nuclear structure facility is under construction, based on a 30 MV tandem Van der Graaf, and work will soon begin on a purpose-built X-ray and ultraviolet source. An IBM 370/165 computer is installed at the Laboratory, and a computational science group has recently been established working mainly on the quantum chemistry area.

The successful applicants will be appointed at salaries between £2901 and £5155, the level at which an associate is appointed depending on age, ability and experience. There are also pay supplements of (a) £313.20 per annum and (b) 5% of total salary subject to a minimum of £130.50 per annum and a maximum of £208.80 per annum. Posts are normally available for a fixed term of three years and are superannuable.

Applicants, male or female, should possess a Ph.D. degree in theoretical nuclear physics, or expect to obtain one during 1978.

Closing date: 1 February 1978

Please write enclosing curriculum vitae, the addresses of two referees and quoting reference number DL/606/T to:

(663)A

SCIENCE
RESEARCH
COUNCIL



DARESBURY LABORATORY

Personnel Officer,
Science Research Council
Daresbury Laboratory,
Daresbury,
Warrington WA4 4AD.

LOTHIAN HEALTH BOARD

Applications are invited for the post of senior grade Biochemist in the Endocrine Unit/Immunology Laboratories in the Royal Infirmary of Edinburgh under the direction of Dr W. J. Irvine. Responsibilities will be primarily to ensure the efficient running of the routine immunology services pertaining principally to auto-immune disease, and for those endocrine laboratory services carried out in the Department, which principally involve in vivo and in vitro tests of thyroid function. Opportunities for research, especially in clinical immunology would be available in collaboration with projects sponsored by the M.R.C. and C.R.C.

Applicants should have a good Honours Degree, and preferably an M.Sc. Ph.D., or first part M.R.C. Path. Experience in immunology is desirable. Whitley Council Conditions of Salary Scale and service apply.

The salary scale is £4,437 to £5,697 plus a salary supplement of £312 per annum, plus Phase II supplement. National Health Service Superannuation Scheme is in operation.

Further particulars on application.

Applications, which should be typewritten, giving particulars of age, qualifications and previous experience, together with the names and addresses of two referees should be lodged with the Secretary, 11 Drumsheugh Gardens, Edinburgh EH3 7QQ, by January 12, 1978.

867(A)

PLANT BREEDER-GENETICIST

ASSISTANT PROFESSOR, available July 1, 1978. Candidates should be prepared to teach undergraduate Plant Breeding/Genetics, develop a graduate Plant Genetics Course, and establish a research programme in his/her own area of specialty. Research experience with corn genetics/breeding is desired. Applicants should send curriculum vitae, transcripts, and three letters of reference to: Dr Hugh Frick, Search Committee Chairman, Plant Science Dept., University of Delaware, Newark, Delaware 19711 before March 1, 1978.

The University of Delaware is an Affirmative Action Equal Opportunity Employer.

838(A)

UNIVERSITY OF NOTTINGHAM DEPARTMENT OF ZOOLOGY

Applications are invited for the post of POSTDOCTORAL RESEARCH ASSOCIATE. The work involves a study of protein reabsorption in the kidney of a lower vertebrate. Candidates should have an interest in electron microscopy, renal physiology and micro puncture techniques.

Salary £3,333 per annum.

Application forms returnable not later than January 11, 1978 from the Staff Appointments Officer, University of Nottingham, University Park, Nottingham NG7 2RD.

898(A)

MEDICAL RESEARCH COUNCIL RADIOBIOLOGY UNIT, HARWELL

SCIENTIST needed to work in a multi-disciplinary group to study the microscopic distribution of inhaled radioactive particles in the respiratory system of experimental animals in relation to radiation-induced carcinogenesis. Preference will be given to candidates who have several years of relevant post-graduate research experience.

The Unit also includes groups studying mutation, genetics, cytogenetics and

Applicants should hold a relevant good honours degree. Starting salary would normally be in the range £3,974-£5,219 according to age and experience. M.R.C. conditions of service would apply. Hostel accommodation for single, or housing for married people may be available. Applications, with curriculum vitae and names of two referees, should be sent to Administrator, M.R.C. Radiobiology Unit, Harwell, Didcot, Oxon. OX11 0RD. Ref. JV/6.

941(A)

UNIVERSITY OF MELBOURNE LECTURESHP (LIMITED TENURE) IN ANIMAL PRODUCTION in the FACULTY OF AGRICULTURE AND FORESTRY

Applications are invited for this position commencing March 1, 1978. The successful applicant will be required to give lectures within the courses of animal production to students in Agricultural and Veterinary Science. He will also be expected to develop a research programme in his field of interest.

An applicant with interest and experience in systems analysis and modelling in animal production is preferred, however, other interests of the section into which he could fit are ruminant nutrition, animal genetics and animal behaviour.

QUALIFICATIONS: Ph.D. or equivalent, with some postdoctoral experience will be preferred.

SALARY RANGE: \$A14,632 to \$19,262.

The position will be offered for a limited tenure of three years. Further information, including details of application procedures and conditions of appointment, is available from The Registrar, University of Melbourne, Parkville, Victoria, 3052, Australia. Applications referring to position 200 199 should be addressed to The Registrar and close on January 31, 1978. Conditions of appointment available from the Association of Commonwealth Universities (Appl), 36 Gordon Square, London WC1H 0PF.

956(A)

THE UNIVERSITY OF MANCHESTER INSTITUTE OF SCIENCE AND TECHNOLOGY LECTURESHP IN CORROSION ENGINEERING/ TEROTECHNOLOGY AND CORROSION SCIENCE

The Corrosion and Protection Centre has a large, varied research school, M.Sc. Courses in Corrosion Science and Terotechnology, an advisory service for industry and provides teaching at undergraduate level. Two appointments are to be made, with some preference for younger candidates (male or female), showing evidence of quality and versatility in the following general fields: (a) Corrosion Engineering/Terotechnology (b) Corrosion Science.

Research areas the Centre wishes to develop further include heat, mass and momentum transfer, design, monitoring, non-destructive testing, economics and the terotechnological approach quantitative modelling, modern experimental techniques, surface coatings and the anodic behaviour of aluminium. Experience in one or more of these subjects would strengthen an application. Commencing salary will be within the scale £3,333 to £6,655 per annum.

Application forms are obtainable from the Registrar, U.I.M.S.T., P.O. Box 88, Manchester M60 1QD, enquiries should be directed to Professor G. C. Wood, Corrosion and Protection Centre (Tel. 061-236-3311 Ext. 2012).

892(A)

FACULTY POSITION IN BIOCHEMISTRY UNIVERSITY OF ILLINOIS AT URBANA CHAMPAIGN

The Department of Biochemistry is seeking applicants for a tenure track appointment at the level of assistant professor or higher to begin August 1978. Applicants should be qualified to teach biochemistry courses at undergraduate or graduate levels. Only candidates who have demonstrated excellence in research and who are prepared to establish an active research program will be considered, preference will be given to those who have received graduate and post-doctoral training in strong research environments. The minimum salary at the assistant professor level is \$15,000 for a nine month appointment; salaries at higher ranks are negotiable. For full consideration, application material, including a curriculum vitae, and list of publications, a brief description of research interests and three letters of reference should be sent by February 1, 1978:

Professor Lowell P. Hager, Head,
Dept. of Biochemistry
University of Illinois
Urbana, Illinois 61801
Telephone: (217) 333 3945

The University of Illinois is an affirmative action/equal opportunity employer.

785(A)

ETH Zürich

The Swiss Federal Institute of Technology (ETH Zurich)

announces a new position for a

Professor of Geodesy

The new professor will be responsible for teaching and research in the fields of mathematical, physical and astronomical geodesy, satellite geodesy and geodynamics.

The candidate must have several years of demonstrated experience and capability in initiating and directing research. He must be willing and competent to provide instruction at all university levels. Special emphasis will be placed on close interdisciplinary cooperation between geodesy and geophysics in both teaching and research.

Applications should be sent with curriculum vitae and list of publications before April 30, 1978 to the President of the Swiss Federal Institute of Technology Zurich, Professor H. Ursprung, CH- 8092 Zurich/Switzerland.

862(A)

SWINBURNE COLLEGE OF TECHNOLOGY

(Melbourne, Australia)

LECTURER IN BIOPHYSICS (Contract Appointment)

Applications are invited from graduates with a relevant higher degree in physiology, clinical biophysics or biomedical engineering and with clinical and/or research experience in cardiovascular physiology. Teaching experience will also be considered in making the appointment.

The appointment will be by contract for three years duration including removal and repatriation expenses. Permanent appointment, during or at the expiration of the period, may be negotiated by mutual agreement.

The successful applicant will be involved in the undergraduate and postgraduate biophysics teaching programme with particular responsibility for the cardiovascular component of these courses. The successful applicant will also be encouraged to participate in an area of relevant applied research.

Swinburne College of Technology is a tertiary educational institution affiliated with the Victoria Institute of Colleges. The Department of Physics is a member department of the Faculty of Applied Science, and has academic responsibility for undergraduate courses in biophysics and instrumental science together with postgraduate programme in biophysics, biomedical instrumentation and instrumental science.

Salary: Lecturer I \$A14,631 to \$16,748; Lecturer II \$A17,145 to \$19,261 p.a.

Applications close **February 18, 1978**. Further information about the position, conditions of employment and application procedure may be obtained from the Association of Commonwealth Universities (Appts), 36, Gordon Square, London WC1H 0PF. 920(A)

FRESHWATER BIOLOGICAL ASSOCIATION WINDERMERE LABORATORY

Applications invited from biologists for two-year contract from April 3, 1978 to work on development of samplers for macro-invertebrates in rivers. Good honours degree essential, preferably Ph.D., experience in river invertebrate taxonomy, field sampling and analysis of numerical data desirable. SCUBA diving experience useful. Post equivalent to Scientific Officer in Civil Service. Gross salary within range £3,078-£4,048. Full details and application forms from Secretary, Freshwater Biological Association, The Ferry House, Ambleside, Cumbria LA22 0LP (Tel: 096 62 2468). Closes February 6, 1978. 942(A)

THE GOVERNMENT OF THE SYRIAN ARAB REPUBLIC ALEPPO UNIVERSITY

ALEPPO UNIVERSITY HOSPITAL

Invites applicants for posts in Anaesthesia vacant 1977/1978. Qualifications: F.R.C.S. one. In addition to five years experience.

Salary: £8,000 to £10,000 per annum; plus £1,350 to £1,700 accommodation allowance per annum.

- One month paid holiday per annum
- Return tickets to country of residence at the end of contract, also for wife and two children.
- Contract for one year renewable.

Please write for full details to:

The Rector
University of Aleppo
Syrian Arab Republic 864(A)

UNIVERSITY OF MELBOURNE CHAIR OF METEOROLOGY (Foundation Appointment)

Applications are invited for appointment to the newly established Chair of Meteorology.

SALARY: \$A31,248 per annum.

Further information, including details of application procedure, superannuation, travel and removal expenses, housing assistance, and conditions of appointment, is available from the Registrar. All correspondence (marked "Confidential") should be addressed to The Registrar, University of Melbourne, Parkville, Victoria 3052, Australia. Information also available from the Secretary General, Association of Commonwealth Universities (Appts), 36 Gordon Square, London WC1H 0PF.

Applications close on **March 31, 1978**. 937(A)

UNIVERSITY OF LEICESTER RESEARCH ASSISTANT IN CHEMISTRY

An S.R.C. Postgraduate appointment is available for a suitable candidate to study "The Aqueation of Alcohols and Sugars using N.M.R. and Infrared Spectroscopy".

The work is a combined study with Professor M. C. R. Symons and Dr W. Derbyshire of Nottingham University, and the initial salary will be up to £3,333 a year, starting as soon as possible.

For details, please contact Professor M. C. R. Symons, Department of Chemistry, The University, Leicester LE1 7RH. 872(A)

UNIVERSITY OF ULM, WEST GERMANY LABORATORY OF IMMUNOLOGY, DEPARTMENT OF MICROBIOLOGY POSTDOCTORAL RESEARCH ASSISTANT IMMUNOCHEMISTRY/ BIOCHEMISTRY

Immunologists are invited to apply for a postdoctoral research assistantship to work on a project concerned with cell interactions in the immune response with special reference to humoral mediators. The position is at least for three years, will be open January 1, 1978 or later and has a salary according to qualification and experience (£12,900 to £13,600). Candidates should preferably have a Ph.D., a background in immunochemistry is essential. Applications including curriculum vitae, a description of research experience, relevant reprints and the names and addresses of two referees should be sent to Dr H.-D. Flad, Laboratory of Immunology, Department of Microbiology, University of Ulm, P.O. Box 4066, D-7900 Ulm, West Germany. 868(A)

KING'S COLLEGE HOSPITAL MEDICAL SCHOOL (University of London)

Denmark Hill London SE5 8RX

Applications are invited for two positions in a busy multi-disciplinary Unit:

- 1 A RESEARCH ASSISTANT, who should be a graduate microbiologist or have equivalent qualifications or experience, to study host defence mechanisms in chronic liver disease. The appointment is for one year in the first instance.
- 2 A RESEARCH FELLOW, preferably post-doctoral with experience in cell membrane biology, immunochemistry or protein biochemistry, to work on human liver cell surface antigens. The appointment is for three years in the first instance.

Salaries and conditions of service according to the University of London Research Assistant's IA/IB scales.

Further details from: Dr T. G. McFarlane, Principal Biochemist, The Liver Unit, Applications to the Secretary of the Medical School not later than January 21, 1978. 919(A)

ECOLOGIST-ASSOCIATE DIRECTOR UCLA

Applications are invited from highly qualified ecologists to become Chief of Division of Environmental Biology and Associate Director of the Laboratory of Nuclear Medicine and Radiation Biology. The position carries a joint appointment, presumably at the full professor level, in the Department of Biology, and undergraduate and graduate teaching responsibilities. Research interests should be in quantitative ecology and applicants should be interested in resolving environmental problems related to energy technologies because the Laboratory's research is funded primarily by the Department of Energy (formerly E.R.D.A.).

Send letters of inquiry or application, including curriculum vitae, description of research and teaching interests, and names and addresses of three references before February 15, 1978 to Director's Office, Laboratory of Nuclear Medicine and Radiation Biology, University of California, 900 Veteran Avenue, Los Angeles, California, 90024. An equal opportunity/affirmative action employer. 902(A)

POSTDOCTORAL POSITION ELECTROPHYSIOLOGY— PHARMACOLOGY

Available immediately, N.I.H. funded. To study nerve cells in tissue culture using voltage clamp and fluctuation analysis with on line computer facilities. Experience in electrophysiology with micro-electrodes essential, computer programming and biophysical orientation desirable. Send CV and references to Dr Fred Sachs, Dept. of Pharmacology, S.U.N.Y., Buffalo, NY 14214, U.S.A. An Affirmative Action/Equal Opportunity Employer. 834(A)

PHARMACOLOGY

We require a graduate with up to 3 years relevant experience to work on the development of drugs for certain cardiovascular diseases. The successful applicant would work within a small team reporting directly to the Team Leader.

The Research and Development Laboratories are situated in the pleasant surroundings of the East Midlands and the position attracts benefits such as flexible working hours, help with relocation expenses in suitable cases, etcetera.

If you are interested please contact Bryan Johnston, Fisons Limited, Pharmaceutical Division, Research and Development Laboratories, Bakewell Road, Loughborough, Leics. LE11 0QY. Telephone 0509 66361 quoting reference number 539R.



945(A)

UNIVERSITY OF MALAWI CHANCELLOR COLLEGE

Applications are invited for the post of
LECTURER IN PHYSICS

tenable as soon as possible. Applicants should have at least a good honours degree in Physics or Electronics. Preference will be given to candidates with a higher degree and whose research interest is in the field of Physics of materials, applied acoustics, solar energy or electronics. Teaching, research and industrial experience would also be an advantage. The appointee will be expected to teach at all levels of the four year general degree programme.

Salary range: K2,809 to K4,714 p.a. The British Government may supplement salary in range £3,450 to £3,864 p.a. (sterling) for married appointees and £2,184 to £2,568 p.a. (sterling) for single appointees (reviewed annually and normally free of all tax) and provide children's education allowances and holiday visit passages. If no British Government supplementation available, university will pay K720 p.a., taxable in Malawi. Family passages, various allowances, biennial overseas leave, gratuity, housing.

Detailed applications (2 copies) should be sent air mail by January 23, 1978 together with curriculum vitae and naming 3 referees to Registrar, University of Malawi, University Office, P.O. Box 278, Zomba, Malawi. Applicants resident in U.K. should also send one copy to Inter-University Council, 90/91 Tottenham Court Road, London W1P 0DT. Further particulars may be obtained from either address.

885(A)

UNIVERSITY OF WISCONSIN-PARKSIDE INDUSTRIAL HYGIENIST

Tenure-track faculty position, September 1978. New baccalaureate program in Industrial and Environmental Hygiene. Rank and salary open and commensurate with experience and qualifications. Candidates must have training and professional experience in Industrial Health and be eligible for board certification. Board certification in Industrial Hygiene is desirable. Appropriate doctoral degree preferred. Well qualified persons with a masters degree may also be considered. Appointee will teach undergraduate courses in Industrial Hygiene, including externships, advise students and participate in university, community and professional activities. We seek candidates who can provide leadership, have strong commitment to teaching, and are involved in research. All replies confidential. Inquiries and applications with three letters of reference to Dr Surinder P. Datta, I.E.H.A. Committee, University of Wisconsin-Parkside, Kenosha, WI 53141 U.S.A. (414-553-2469), by February 20, 1978. An affirmative action/equal opportunity employer. 716(A)

UNIVERSITY OF OXFORD Microbiology Unit DEPARTMENT OF BIOCHEMISTRY POSTDOCTORAL

RESEARCH ASSISTANT—Ref. B14

There is a vacancy for a postdoctoral biochemist or microbiologist to work on bacterial sporulation. The appointment, which will be for one year in the first instance, on the scale £3,333 to £5,627, may be extended for a further period of two years and there is provision for U.S.S. membership.

Applications, which should include relevant details and the names of two referees, should be sent before February 28, 1978, to Professor J. Mandelstam, Microbiology Unit, Dept. of Biochemistry, South Parks Road, Oxford OX1 3Q.

DEPARTMENTAL DEMONSTRATORSHIP—Ref. B15

It is proposed to appoint a Departmental Demonstrator from October 1, 1978. The salary will be on the scale £3,333 to £3,833, according to qualifications and experience and with membership of U.S.S. The appointment is for a period not exceeding 3 years. The successful candidate will be expected to take part in departmental teaching and to conduct his or her own research.

Applications (3 copies) including the names of two referees, should be sent to: The Administrator, Department of Biochemistry, South Parks Road, Oxford OX1 3QU not later than February 28, 1978. 863(A)

OXFORD UNIVERSITY

THE UNIVERSITY:
ST. EDMUND HALL

and

ST. HUGH'S COLLEGE

Nuffield Medical Research Fellowship 1978

Applications are invited for a Nuffield Medical Research Fellowship to be held in conjunction with a Junior Research Fellowship at one of the above colleges. The Fellowship will be tenable for two years from April 1, 1978 (or a later date by agreement) in one of the clinical medical departments of the University and be renewable for a third year. Scale of stipend will be £4,665-£5,643 p.a. if medically qualified and £3,333-£4,190 p.a. if not.

Further particulars of the Fellowship may be obtained from The Secretary, Board of the Faculty of Clinical Medicine, 1A Observatory Street, Oxford OX2 6EW, to whom applications, together with the names of two referees should be sent by January 31, 1978. 943(A)

WELSH NATIONAL SCHOOL OF MEDICINE

(University of Wales)

Applications are invited for the post of
POSTDOCTORAL

RESEARCH IMMUNOLOGIST

at the K.R.U.F. Institute of Renal Disease, Cardiff. This post is available under a Programme Grant from the M.R.C. It will be for one year in the first instance and may be renewable annually thereafter for up to three years; commencing salary, according to qualifications and experience, in the region of £4,600. The successful applicant may initiate his own programme of research but will be expected to assist in the immunological evaluation of kidney recipients and the assessment of new immunosuppressive regimes. Ample laboratory space is available within the Institute. Applications, which should take the form of a brief curriculum vitae with the names and addresses of two referees, should be sent to the Registrar, Welsh National School of Medicine, Heath Park, Cardiff, quoting reference No. M6/7/14. Further information available from Mr John K. Salaman, M.Chir., F.R.C.S., tel. no. 0222 492233 ext. 450, 870(A)

UNIVERSITY OF CAMBRIDGE DEPARTMENT OF PHYSICAL CHEMISTRY POSTDOCTORAL RESEARCH ASSISTANT

Applications are invited for a Postdoctoral Research Assistantship in the Department of Physical Chemistry for research into aspects of two-photon absorption spectroscopy, particularly the study of Inverse Raman Spectra. Experience in the development and construction of high power visible lasers would be of value, as would a background in high resolution molecular spectroscopy and/or electronics. The post is tenable for a period of one year, but there may be a possibility of an extension for a second year. The initial salary will be within the range £3,333 to £3,547 per annum according to qualifications and experience (appointments outside this range may also be considered) plus U.S.S. benefits.

Applications, stating age, academic qualifications and experience, together with the names of two referees, should be submitted to Dr W. J. Jones, Department of Physical Chemistry, Lensfield Road, Cambridge CB2 1EP. 933(A)

FIELD STUDIES COUNCIL

TUTOR in ECOLOGY at Juniper Hall Field Centre, Dorking, Surrey. Single, good degree in biology or other appropriate science, botanical interest and experience of lowland terrestrial ecosystems and knowledge of French or German an advantage. Educ. qualif. desirable. Salary £1,944 by 84 by 84 to £2,112 plus free board and lodging. Appointment February 1978 if possible. Closing date for applications January 20, 1978. Further details and application forms from The Director, Field Studies Council, Preston Montford, Montford Bridge, Shrewsbury SY4 1HW. 914(A)

THE CITY UNIVERSITY DEPARTMENT OF CHEMISTRY Lecture Course on THE FLAMMABILITY AND BURNING OF SOLID MATERIALS

A course of postgraduate or post-experience lectures, arranged by Professor J. H. Burgoyne, will be given from March 6-8, 1978. This Course will be concerned with the mechanisms of ignition, burning and retardancy of solid materials and will attempt to relate this understanding, through testing and legislative control, to the practical use of these materials.

Further details may be obtained from the Secretary of the Chemistry Department, The City University, St John Street, LONDON EC1V 4PB. Telephone 01-253 4399 (extension 532). 603(D)

NATO ADVANCED STUDY INSTITUTE Interlinking of Computer Networks

BONAS, FRANCE August 28th to September 8th, 1978

This Institute offers a two-week structured programme designed to investigate in depth the problems inherent in the transmission of data between and through computer networks.

Acknowledged experts drawn from the international scientific community will provide extensive reviews of the technical and organisational issues involved. The lectures will be supported by tutorial discussions and work-shops.

Approximately sixty scientists will be selected to attend the Institute.

A certain number of supporting NATO fellowships are available to participants from membership countries.

Fellowships will be considered on the basis of individual merit and fair distribution among membership countries.

Lecturers include:

J. W. Burden (U.K.), V. Cerf (U.S.A.), D. W. Davies (U.K.), H. J. Helms (Italy), P. Kirstein (U.K.), F. F. Kuo (U.S.A.), G. Le Moli (Italy), T. Pyke (U.S.A.), E. Raubold (Germany), H. Zimmerman (France).

Forms of application are available from:

Dr K. G. Beauchamp, Director, Computer Services Dept., University of Lancaster, Bailrigg, Lancaster, U.K. Telephone: (0524) 65201

The deadline for receipt of completed applications is March 1st 1978. 901(K)



LOUGHBOROUGH UNIVERSITY OF TECHNOLOGY

GEL FILTRATION AND ELECTROPHORESIS

A one week residential course from April 3 to 7, 1978, on the theory and applications of gel filtration, ion-exchange, electrophoresis on cellulose acetate, polyacrylamide and other media, isoelectric focusing and isotachopheresis, affinity and hydrophobic chromatography, and immunoelectrophoretic techniques. There will be ample opportunities for course members to run their own samples and discuss problems with representatives of leading manufacturers in these fields. The course is suitable both for beginners and for more advanced workers.

The course fee is £100 including accommodation. Applications and further information from Dr J. N. Miller, Department of Chemistry.

Next course in the series:
Spectrofluorimetry and Related Methods.
July 3 to 4, 1978.

Loughborough Leicestershire
883(D)

FELLOWSHIPS

RUHR-UNIVERSITY BOCHUM (Germany)

Applications are invited from suitable qualified candidates for a

RESEARCH FELLOWSHIP IN CELL PHYSIOLOGY

The position, which is financially supported by the Deutsche Forschungsgemeinschaft (SFB 114) is connected with the Department of Cell Physiology, Ruhr-University Bochum. The successful candidate would be expected to work in one of the following fields: **Electrophysiology of skeletal or heart muscle cells or synapses, excitation contraction coupling, pores in artificial membranes.**

The salary ranges from 1,700 to 3,500 DM per month, after deductions (depending on qualification, age, marital status etc.). Travel expenses can in addition be paid.

The appointment will be for one year, it can be prolonged for up to three years.

Applications including a curriculum vitae and the names of two referees should be sent to

Prof. H. G. Glitsch or Prof. H. Ch. Lüttgau
Department of Cell Physiology
Ruhr-University Bochum
P.O. Box 102148
D-4630 Bochum 1, F.R.G. 875(E)

THE UNIVERSITY OF LEEDS DEPARTMENT OF MECHANICAL ENGINEERING I.C.I. RESEARCH FELLOWSHIP ON BEARING INFLUENCED ROTOR DYNAMICS

Applications are invited for a 3 year, I.C.I. sponsored, Research Fellowship. The work involved represents a co-operative project on bearing influenced rotor dynamics between I.C.I. and the University of Leeds; it will include the development of computer programs to predict the vibrational characteristics of rotor/bearing systems, and the application of the programs to particular problems of interest to the sponsor. Experience in system dynamics and/or bearing analysis would be an advantage. The Research Fellow, who will be based at the University of Leeds but will also carry out investigations in industry with I.C.I. staff, may be a candidate for a higher degree, or a Postdoctoral Fellow. The salary will be on either the 1A Scale for Research and Analogous Staff £3,333 to £5,627; but in the case of a candidate with exceptional qualifications and experience a higher figure may be negotiable.

Applications, and further enquiries, should be directed to Professor D. Dowson, Department of Mechanical Engineering, The University, Leeds LS2 9JT (Tel. 0532-31751 ext. 254). 950(E)

UNIVERSITY OF EAST ANGLIA Norwich POSTDOCTORAL FELLOWSHIP (S.R.C.)

in the School of Biological Sciences for three years to investigate a hybrid zone in an alpine grasshopper. This will involve a major study of allozyme variation, which will be complemented by genetical and ecological studies. Initial salary in the range RA (1A) £3,333 to £3,761.

Applications with curriculum vitae and naming three referees as soon as possible to Dr G. M. Hewitt, School of Biological Sciences, University of East Anglia, Norwich NR4 7TJ, from whom further information is available. 815(E)

SENIOR RESEARCH FELLOW IN ATOMIC/MOLECULAR PHYSICS

The University of Maryland and the National Bureau of Standards in Washington, D.C. are announcing a one year fellowship for a senior research scientist to pursue research at the N.B.S. Synchrotron Ultraviolet Research Facility (S.U.R.F.). The S.U.R.F. fellow will hold an appointment in the Institute of Physical Science and Technology at the College Park Campus of the University of Maryland. In addition to his research activities at S.U.R.F., the appointee will interact with the faculty and students at the University, principally through lectures and seminars.

Applications are now being accepted for the academic years 78-79 and 79-80, with detailed schedule to be negotiated. Salary and faculty rank will be commensurate with current position. We are initially seeking an individual interested in pursuing atomic/molecular physics at S.U.R.F. Experience in the use of synchrotron radiation and/or photoelectron spectroscopy of gases will be considered particularly valuable for the 78-79 appointment. Later appointments may be in other fields.

Send résumé, desired starting date and research plans to:

Prof. Joseph Silverman
Institute for Physical Science and
Technology
University of Maryland
College Park, Maryland 20742 954(E)

UNIVERSITY OF MELBOURNE JUNIOR RESEARCH FELLOW DEPARTMENT OF MATHEMATICS

Applications are invited for a post-doctoral Junior Research Fellowship in the Department of Mathematics from candidates with suitable qualifications in statistical mechanics. The Fellowship is funded by an ARGC grant for a project titled "Statistical Mechanics of Liquids and Liquid-Solid Interfaces". The appointment is for a period of up to two years and includes a contribution towards travel expenses.

SALARY: \$A12,160 to \$A13,390 per annum.

The appointment will commence as soon as possible.

Enquiries may be directed to Dr E. R. Smith, Department of Mathematics.

Further information, including details of application procedure is available from The Registrar. Applications referring to Position Number 618333 should be addressed to The Registrar, University of Melbourne, Parkville, Victoria, Australia, 3052. Closing date for applications is February 28, 1978.

889(E)

DEPARTMENT OF MICROBIOLOGY DALHOUSIE UNIVERSITY HALIFAX, NOVA SCOTIA, CANADA

Applications are invited for POSTDOCTORAL FELLOWSHIPS to study viral pathogenesis with special emphasis on the role of interferon. Fellowships beginning July 1, 1978, are available for a period of two years with an initial stipend of \$12,720 per annum. Candidates should have research experience in the field of animal virology, preferentially with an interest in the biochemistry of nucleic acid biosynthesis. Applications, consisting of curriculum vitae and names of two referees, should be sent not later than March 30, 1978 to Dr K. R. Rozee, Professor and Head, Department of Microbiology, Faculty of Medicine, Dalhousie University, Halifax, Nova Scotia, Canada. 932(E)

JESUS COLLEGE, OXFORD

The College invites applications from men and women for a Junior Research Fellowship open to candidates intending to pursue research in any branch of Chemistry and tenable for 2-3 years from October 1, 1978. Further particulars may be obtained from The Principal, Jesus College, Oxford OX1 3DW, to whom applications must be sent no later than January 31, 1978. 897(E)

SYMPOSIUM

2nd International Symposium on

QUANTITATIVE MASS SPECTROMETRY IN LIFE SCIENCES GENT, JUNE 13-16, 1978

Contact Professor A. De Leenheer

Laboratoria voor Medische Biochemie en Klinische Analyse

GENT, BELGIUM

(602)M

FOURTH EMBO ANNUAL SYMPOSIUM

3rd-7th May, 1978

"MOLECULAR NEUROBIOLOGY"

The Symposium will be held at the European Molecular Biology Laboratory, Heidelberg, which is to be opened on Friday, 5th May, by the President of the Federal Republic of Germany.

Topics covered at the Symposium will include neuroreceptors, neuropeptides, development of the nervous system, neutral cytoskeletons, higher brain functions.

Plenary Sessions: The speakers will include

B. Boycott (England), D. Bray (England), M. Brownstein (USA), J.-P. Changeux (France), M. Cuénod (Switzerland), G. Edelman (USA), S. Fuchs (Israel), D. Gilbert (England), J. Glowinski (France), B. Hamprecht (Germany), E. Helmreich (Germany), J. Heuser (USA), T. Hökfelt (Sweden), J. Jansen (Norway), M. Lazdunski (France), R. Levi-Montalcini (Italy), U. Littauer (Israel), J. Massoulié (France), E. Neher (Germany), Ph. Nelson (USA), L. Olson (Sweden), W. Reichardt (Germany), M. Schramm (USA), W. Singer (Germany), J. Stinnakre (England), N. Strausfeld (Germany), L. Terenius (Sweden), H. Thoenen (Switzerland).

Poster Sessions

In addition to the plenary sessions poster sessions will be held each day during the Symposium to allow participants to present contributions on topics related to or complementing the plenary sessions as well as on recent technical developments.

Participation

Applications should reach Dr. J. Tooze, Executive Secretary, European Molecular Biology Organization, Postfach 102240, 69 Heidelberg 1, West Germany, before the end of February. They should include a curriculum vitae, an indication of the applicant's scientific background, and current research interests. Participants who wish to contribute a poster should submit a title and an abstract of 1-2 pages. A registration fee of DM 80 will be charged. Bed and breakfast and accommodation (DM 20-40 per day) in the vicinity of the EMBL will be arranged by the Symposium secretariat. Participants will be responsible for their living and travel expenses.

953(M)

THE UNIVERSITY OF MANCHESTER INSTITUTE OF SCIENCE AND TECHNOLOGY

Chemical Society Macromolecular Group
Society of Chemical Industry Plastics and
Polymer Group

Polymeric Biomedical Materials

A Symposium to be held in Manchester on
Tuesday and Wednesday 18th and 19th April 1978

The aim of this Symposium is to inform polymer scientists of the requirements which polymeric materials must fulfil in biomedical applications but it may also be of interest to medical scientists by indicating how improved materials might be developed which would meet requirements better than those currently available.

The Introductory Lecture will be given by Professor D. F. Gibbons (Case-Western Reserve University, USA). Subsequent sessions will discuss *Polymers for Tissue Replacement* (Speakers: Mr D. Annis (Liverpool), Professor M. Braden (London), Dr G. W. Hastings (Stoke), Mr G. P. Pearson (Bradford), Dr G. D. Winter (Stanmore)); *Polymers as Biomedical Materials* (Professor A. Bantjes (Holland), Professor E. P. Goldberg (Florida), Professor N. Graham (Strathclyde), Dr A. Rembaum (California)); and *in vitro Testing of Polymers for in vivo Applications* (Dr G. Braun (Ruhchemie AG), Dr J. M. Courtney (Strathclyde), Dr W. S. Haworth (Oxford)).

Further details may be obtained by application to
The Registrar, UMIST, P.O. Box 88,
Manchester M60 1QD

908(M)

HERIOT-WATT UNIVERSITY

DEPARTMENT OF CHEMISTRY

S.R.C. POSTDOCTORAL RESEARCH ASSOCIATESHIP

Applications are invited for a Post-doctoral Research Associate to work on the development and application of ¹⁸¹Ta Mössbauer spectroscopy. The successful applicant is likely to be a chemist or physicist with an interest in and preferably experience of research in solid state science and/or Mössbauer spectroscopy.

The post is tenable for two years on salary range 1A (up to £3,547 p.a. plus U.S.S. membership in the first instance).

Applications, with curriculum vitae and the names of two referees, should be sent as soon as possible to Dr M. J. Tricker, Department of Chemistry, Heriot-Watt University, Edinburgh EH14 4AS from whom further information may be obtained.

905(O)

QUEEN MARY COLLEGE

University of London

CHEMISTRY DEPARTMENT

Applications are invited for a

POSTDOCTORAL

RESEARCH ASSISTANTSHIP

supported by Hoffmann-La Roche to investigate the structure of caroteno-proteins by the synthesis and recombination of modified carotenoids and a study of the physical properties of the products. Salary in range £3,805 to £4,233 p.a. (under review). Please send curriculum vitae and names of two referees to The Registrar (N), Queen Mary College, Mile End Road, London E1 4NS.

890(P)

GRADUATE ASSISTANTSHIPS

are available for well qualified pre-doctoral students in Geophysical Fluid Dynamics, particularly in the area of computer and laboratory modelling of variations in the climate. A strong background in mathematics and physics is needed but prior training in a geophysical discipline is not necessary. Minorities and women are especially encouraged to apply. Write to Director, Geophysical Fluid Dynamics Institute, 18 Keen Building, Florida State University, Tallahassee, Florida 32306.

784(P)

ANNOUNCEMENT

The International Congress of Inflammation, sponsored by the Institute of Pharmacology and Pharmacognosy of the University of Milan, will take place in Bologna (Italy) on October 31/November 4, 1978 under the chairmanship of professors B. Samuelsen, G. Weissmann, R. Paoletti.

The Congress will be divided into the following sessions: Biology, Preclinical Pharmacology of the Anti-Inflammatory Agents, Clinical Pharmacology of the Anti-Inflammatory Agents; special emphasis will be given to the clinical applications and side effects of anti-inflammatory agents on specific organs and systems.

For information and programs please write to: Dr E. Folco—F.G.L.—Via Monte Napoleone, 23-20121 Milan (Italy)—Tel. 702 267 and 783 868.

906(N)

International Union of Forest Research Organisations

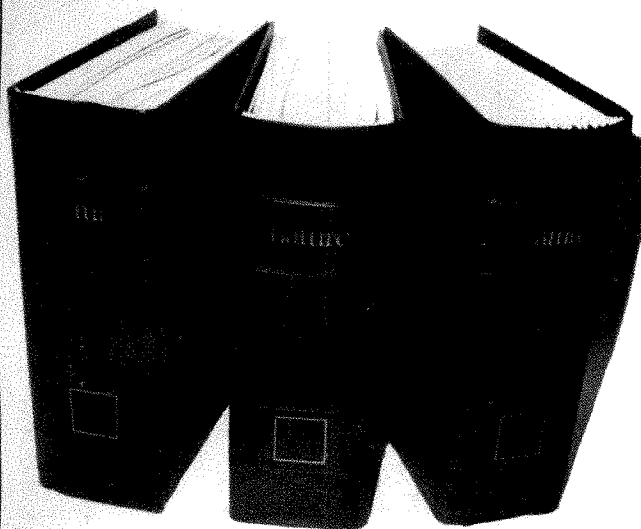
A MEETING on

GENETICS OF HOST-PARASITE INTERACTIONS IN FORESTRY

will be held in Göttingen in September 1979. There will be not more than five days devoted to formal sessions and demonstrations of research work. Some field tours will be included. For more information contact Dr Kim von Weissenberg, Finnish Forest Research Institute, SF-77600 Suonenjoki, Finland.

910(G)

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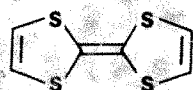
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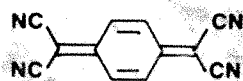
TTF and TCNQ

Components for conductivity

Tetrathiafulvalene (TTF) and several other tetrathioethylenes were originally investigated as possible electron-rich olefins.¹ It was quickly realized that the electrochemistry of TTF was by far the most interesting aspect of the compound. Wudl *et al.*^{2,3} discovered that TTF formed an exceptionally stable radical cation complex with chlorine (TTF⁺Cl⁻) which exhibited an unusually high electrical conductivity.



TTF

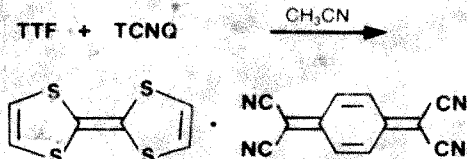


TCNQ

7,7,8,8-Tetracyanoquinodimethane (TCNQ) was first studied for its ability to form radical anions.^{4,5,6} Since then, many practical applications have been discovered. For example, TCNQ is used in the:

- 1) colorimetric determination of free radical precursors⁷
- 2) visualization of certain nitrogen and sulfur compounds on thin-layer and paper chromatograms^{7,8}
- 3) replacement of MnO₂ in aluminum solid electrolytic capacitors⁹
- 4) construction of heat-sensitive resistors¹⁰
- 5) induction of radical polymerizations (in combination with *N,N*-dimethylaniline *N*-oxide)¹¹
- 6) construction of ion-specific electrodes.^{12,13}

It was the ability of TCNQ to form radical anions that prompted Cowan¹⁴ to combine it with the electron donor TTF. The resulting charge-transfer complex was found to contain TTF and TCNQ in a 1:1 ratio.



This complex behaves electrically and optically like a one-dimensional metal at room temperature. It has one of the highest electrical conductivities known for an organic compound, being highly anisotropic along an axis defined by the colinear stacks of TTF and TCNQ.¹⁵ Since there was

some controversy over the exact value of the conductivity, a study was performed to determine if the chemical purity of the components affected the electrical conductivity of the complex.¹⁶ The workers concluded that crystal perfection rather than chemical purity was the factor chiefly responsible for determining the degree of conductivity. Major research efforts are currently in progress to better understand and find applications for the unusual properties of the TTF/TCNQ complex.^{17,18,19}

Aldrich has offered TCNQ for many years. Now we also offer TTF! With the ready availability of these "components for conductivity," the TTF/TCNQ complex is more accessible for further studies.

References:

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Registered as a newspaper at the
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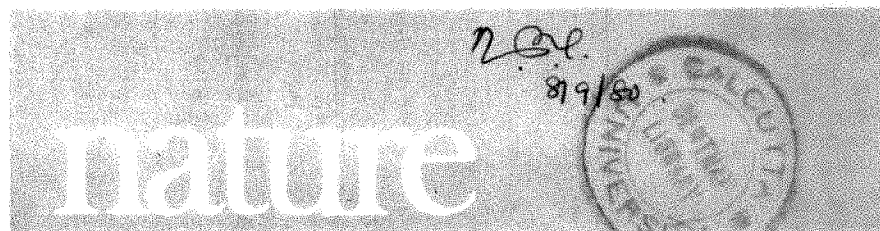
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12 January 1978

Vietnam—an inexpensive form of help

RECENTLY Dr Alastair Hay, a nutritional biochemist who has written frequently in our news pages over the past few years, went on a visit to Vietnam with financial support from *Nature*. The first of his three reports on postwar Vietnam appears in this issue. It comes at a time when Vietnam finds itself yet again involved in conflict, this time with its neighbour Kampuchea (Cambodia) over borders, although there is as yet no clear indication that the skirmishes will have a serious effect on redevelopment within the war-weary nation.

The scientific tradition has not been particularly strong in the past in this part of Asia; certainly not as strong, say, as in the Indian sub-continent or China. True, no country has ever had so much sophisticated technology dropped on it or so many large-scale and sinister 'experiments' performed in it, such as weather modification, the automated battleground, and defoliation. But all of this is of little value in postwar days and if Vietnam is to emerge as a healthy nation it will have to use its relatively few scientists intelligently and to give science a high priority in development plans. This it seems to be doing quite well.

But there are pitfalls along the way. The view has been expressed that since the army of a relatively small country was able to contain all the fury of the United States so might Vietnam's small band of scientists do remarkable things on their own. This is not, it should be said, a view expressed by the scientists themselves. But it has a certain superficial appeal and could do a lot to harm the development of good science within Vietnam. For there are already forces at work trying to make Vietnam stand on its own

feet economically—the USSR, the largest supplier of aid to Vietnam and also the country with the largest influence, is cutting back its aid in a drive to make the country more self-sufficient. If scientists, too, sense that they are being required to go it alone they could face grave difficulties.

For what Vietnamese scientists clearly need more than anything else—more even than gifts of expensive equipment, more than expressions of appreciation from politicians—is normal scientific relations with the rest of the world. This would mean a much greater flow of books and journals into the desperately under-equipped libraries and, even more, a flow of people into and out of Vietnam. And it is particularly important that Vietnamese scientists should not feel constrained to establish good relationships only with those in the West whose political position on the war was a matter of public knowledge. For sure, a small number of such scientists have done a lot of good for Vietnamese science, and have helped to raise Western awareness of the Vietnamese plight, but now the net has to be cast much broader to take in anyone who might make a significant contribution, whether or not they are politically committed.

The largest obstacle to the development of science in Vietnam is an information barrier—not deliberately erected but just a natural consequence of war and isolation. It needs a concerted effort by all scientists of good-will to dismantle it. In Britain the Ministry of Overseas Development, the British Council and the Royal Society are all in a position to make a contribution to this relatively low-cost way of stimulating science. □

Holiday diary

January 7. To the misnamed Science Museum at South Kensington with the children. After the tide of irrationality and anti-science of the past decade, good to see the place filled with youngsters still interested in logical ways of thinking. Sure, most of them will remember the place mainly for the knobs to press and the handles to turn, but even so something probably rubs off on them of fascination for machinery and orderliness. 'Please keep off the moon', says the lunar exhibit. Lasers all the rage these days; one on the roof of the Royal Academy, of all places, to advertise a show there; one used at Covent Garden in Tippett's new opera; so off to top floor to see Science Museum's own Laser exhibition, even though pricey to go in (75p). Quaint

and dubious distinction between 'laser light' and 'ordinary light' on a display. Lots of good honest mundane uses of lasers—communication, pipe laying, surveying, cutting cloth and so on. Striking Russian holograms of museum-pieces. Children a bit bored because not many buttons to press. Quite a surprise to get to the end of the show so quickly, though children clearly mildly relieved as they can get back to the working models. Holograms on sale at the exit, for £12.50 each. But what do they portray? 'That's Paul Revere', says man at desk. 'Who?', says my neighbour. 'Haven't you anything else?', he asks, 'Yes—we have all the astrological signs too'. Rapid exit to the irrational world. □

Disaster relief needs more research

John Rivers, of the London Technical Group, argues that more research on disaster relief is needed if funds are to be used effectively*

THE CYCLONE and tidal wave that struck Andhra Pradesh in November last year killed about 30,000 people. But it devastated half a million, and made 2,000,000 more homeless. In the previous two years cyclones had also struck the state, ruining the harvest and leaving farmers deeply in debt. So, at the start of 1978, Andhra Pradesh is poised between recovery and a collapse that would make it rank alongside Sahel and Ethiopia in the vocabulary of suffering.

The immensity of this disaster has not, however, been reflected in the interest shown in it by the rest of the world. It has been a minor story, a few column inches on an inside page, while UK headlines have concentrated on the firemen's strike and the value of the dollar. Few seem to care about Andhra Pradesh. USAID figures, issued one week after the cyclone, estimated that aid from the international community was less than 1% of the \$675,000,000 of damage done. In Britain, the Disaster Emergency Committee's Appeal was a flop, raising only a quarter of the desired million pounds.

The public's lack of interest may not be surprising. The media have produced an unending diet of catastrophe. And the public may be becoming cynical about the usefulness of aid. This would be understandable, given such misdirected aid as the sending of skis to flood victims or brassieres to the starving, which rightly get pilloried in the press. But though newsworthy such events are of minor importance. The major waste in relief funds is more subtle.

In the rare cases where disaster research has been undertaken, the research has exposed the mythological basis of traditional relief. Inoculation campaigns, for example, have been an expensive component in all flood relief programmes since the war, as they were in Andhra Pradesh. Yet epidemiological studies by the Centre for Disease Control, Atlanta, Georgia, have revealed them to be unnecessary and ineffective. The vaccines are not as efficient as is often supposed, and they take about 10 days and two or more inoculations to give a reasonable level of immunity. Moreover the dilution of sources of contamination is much greater, and disruption of water supplies much less in disasters than has hitherto been supposed. So the alternative of simple symptom-reporting systems and intensive investigation of supposed outbreaks, is, as the Guatemalan experience has shown (Romero, A. B., Cobar, R., Western, K. A. & López, S. M., *Disasters* 2, 19-26; 1978) cheaper and more efficient than the fabulously expensive vaccination campaigns that hypnotise relief agencies. The money wasted on such unnecessary flummery is unlikely to have saved lives in Andhra Pradesh; almost certainly it cost lives by diverting scarce resources from more useful action. Other examples of such re-evaluation could be cited, even more await study. As long as disaster aid is less than adequate there will be a need for a science of relief, for a disaster technology.

In the days following the Bengal cyclone in 1971, the London Technical Group (LTG) was started by a group of postgraduate students anxious to encourage the growth of this field. Our first inclination was to initiate a dialogue between aid administrators and academic scientists. It was a twofold failure. First, most academic scientists had a crucial problem in their lack of practical experience of disaster situations. Scientific expertise, no matter how exalted, could be proved inappropriate by peculiarities, often

social, of the population affected by the disaster. Food aid, for example, is a fairly unchallenged item of disaster relief, being sent on the premise that if infantile malnutrition is endemic in good times, it must reach horrific proportions following a disaster. In Andhra Pradesh, as in the Bengal Cyclone, this is unlikely: the sea surge itself killed the very young and the very old, so that the malnourished and vulnerable died, and only the fittest survived. The aid sent presupposed high levels of infantile malnutrition; they are probably lower than ever before.

In housing too the social factor can negate apparently good logic, and the whole field of disaster shelter is littered with clever designs that nobody wants as homes. There is a considerable body of evidence that the best choice for disaster shelter is to use local materials and local labour to rebuild. The result is acceptable, familiar, and morale boosting (Davies, I, *Disasters* 1, 82-90; 1977). Most architects and designers, however, seem to be unaware of these constraints, and find ready sponsors for their designs in government and charitable disaster relief agencies.

This pattern is repeating itself in Andhra Pradesh where the state government and some voluntary agencies are erecting pre-stressed concrete buildings ill-suited to the climate but enough of a status symbol to lead to consumer resistance against the modifications of traditional buildings that are being offered by other agencies. This indeed is typical of the second reason for LTG's failure:—the unwillingness of voluntary agencies and governments to accept scientific evidence, where this points to the need for a change in accepted practice.

In view of this failure it became apparent that if scientists are to contribute to disaster research, there must be a re-assessment of the roles they play.

- First, acknowledging the limitations of the individual expert advisor, we should set out to develop multidisciplinary groups who can meld their individual talents into a new discipline of disaster research. UNESCO already has its earthquake team which visits the site of earthquakes for seismic research. There is no reason why analogous teams, independent of aid donors, should not be formed to investigate the social and medical impact of natural disasters. Research into the effectiveness of disaster aid could be immediately undertaken if only the aid agencies would agree to fund it. It would provide valuable evidence about any need for change in aid policies. In time, if such research became routine, results would be available to relief agencies to direct aid in any current disaster, rather than on a retrospective basis.

- Second, there is a need for longer term investment in research into the nature of disasters themselves. There is much we need to know about disasters, and the social response to them. Scientists as yet may have only an impersonal understanding of disasters, but at least they do know how to ask questions.

As always with research the problem is one of money, and investment in a programme of disaster research requires an act of faith by some funding agency. Justification for such faith may be found in the surprising yield of results from the fragmentary funding the subject has received in the UK and USA over the last 10 years—sufficient indeed to support two journals specialising in the field.

What can science achieve? Eloquent testimony exists in 'Maladie de Famine', a little known book of researches into the pathophysiology of famine which, although over 30 years old, is still the definitive work on the topic. It was a study conducted in the Warsaw Ghetto, by scientists, themselves victims of starvation and under sentence of death. No better memorial could be found to these men than to continue their work. □

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Science helps to rebuild Vietnam

Vietnam believes science to be the key to economic recovery. She hopes to have a "modern scientific capability" within 15 years. **Alastair Hay** reports

ONE of the longest wars in this century, the war in Vietnam, finally came to an end on 30 April 1975. It had claimed over a million lives and was fought for the best part of thirty years.

Few countries can have featured in as many television documentaries as Vietnam. For many of those watching, the image of Vietnam was fashioned by the television screen; it left a memory of a battered people and scarred countryside.

This is the legacy of war. Vietnam faces innumerable problems as she begins her programme of reconstruction. The problems in the health field illustrate the extent of the damage. The World Health Organisation cites figures from Vietnam of one million people infected by venereal disease, about 300,000 prostitutes, and half a million drug addicts, in a population of some 50 million. Endemic malaria affects some 18 million people in Vietnam. And the annual tuberculosis mortality rate is 30 per hundred thousand—the highest in the western Pacific.

Agricultural problems are just as daunting. Vietnam feeds 50 million people, but only farms 5 million hectares. Comparable figures for Eastern Europe cited by Vietnamese agriculturalists are estimated at a ratio of one person per hectare. If Vietnam is to become self-sufficient in food production—which is her intention—her tillage has to increase, and the efficiency of crop cultivation must improve.

But two obstacles impede her progress; unexploded weapons and bad weather. Literally hundreds of square miles of agricultural land have to lie fallow because of the presence of unexploded bombs and mines; mine-sweeping—currently being undertaken by the army—is required before the land can be pronounced safe.

The weather, too, has had disastrous consequences for the last two rice harvests. First it was too dry and then too cold. Yields have been too low to meet home demand and monthly rice rations have had to be reduced from 18 to 14 kilograms per person per month. To make up the remainder of the shortfall, scarce foreign currency had to be used to purchase rice on the international market. That currency was to have bought 3,000 tractors to aid the mechanisation of agriculture. In the event only 500 could be



Suffering from the ravages of war

acquired, all of which are in use in the Mekong Delta—the country's most fertile region. 500 tractors for a population of 50 million people can hardly be expected to revolutionise Vietnamese agriculture.

It is against this background that the scientists in Vietnam are having to orientate their work. They may lack the necessary material resources to tackle many of the problems confronting the country, but they have no lack of prestige. Science is seen by the Government as the key to Vietnam's economic recovery. Science and technology are important enough to warrant a spokesman in the Politburo—the highest policy making body. The minister with this responsibility is General Vo Nguyen Giap.

Giap is not well known in the scientific world. As his title might suggest, his has been a military career. Giap's claim to fame is as victor of Dien Bien Phu, the battle which in 1954 signalled an end to French involvement in Indochina. He has had a number of other responsibilities before assuming the portfolio of Minister of Defence, a post he still retains.

Vietnam's scientists were not displeased when Giap was nominated to deal with their problems. They find him an able minister and effective spokesman. To have one of the seven members of the Politburo—and a possible future prime minister—as their

representative is a situation any lobby would welcome.

Much of Vietnam's basic research is done in the Vietnamese Scientific Research Centre (VSRC) in Hanoi. The centre, now nearing completion, is part of the Soviet Union's aid programme to Vietnam. It will eventually house over 1,000 scientists, technologists and technicians in its four component institutes: those of biology, chemistry, physics and earth sciences. Two additional institutes (of oceanography and experimental biology) are located in the south of the country in Ho Chi Minh City (formerly Saigon).

Dr Nguyen Van Dao is the most senior of the seven vice-chairman of the VSRC; in effect he is its director. In an interview made easier with numerous cups of tea, many bananas and sweets he outlined his centre's research programme. The centre is, he says, "the main research institution in Vietnam". It also controls some Government programmes. One of the most important, says Dao, "is an investigation of our natural resources". Dao considers this knowledge to be vitally important for a developing country. The scientists in the centre will be surveying the coastal seabed as well as mapping land resources. Dao adds that many Vietnamese scientists

Alastair Hay was recently in Vietnam as a Nature travelling fellow. He was supported in part by the London Technical Group.

suspect that the United States could have valuable satellite pictures which would aid the surveys they propose to make. He acknowledges, however, that this information will probably be classified, but maintains that the Vietnamese would make better use of it than the Americans.

The VSRC, says Dao, is really only starting its research programme; its responsibility will increase in the future. Emphasis is currently placed on the problems of agriculture and medicine; on developing the country's electronics industry; and on establishing geophysics and hydrodynamics on a sound basis. "Our aim" says Dao "is to have a modern scientific capability in 15-20 years. This is a great responsibility. We are virtually starting from scratch after 30 years of war and at a time when the country is so short of resources". Dao would welcome extensive collaboration with Western scientists and scientific institutions. This, together with donations of scientific literature and equipment, could help Vietnam meet her twenty year programme. Dao expressed his appreciation for the aid the centre has received from other socialist countries, but adds that there is a desperate need for much more help.

On a tour of the centre it is apparent that some subjects are better provided for than others. Physics is generally acknowledged to be an expensive science, chemistry less so, but costly nevertheless. Historically this is how resources have been apportioned in Vietnam for both physicists and chemists were needed in the war effort. Biology and the other sciences all trailed behind.

The Head of the Institute of Biology, Dr Tuoc, has hopes that this situation is about to change in view of the Government's stated view that agriculture must be accorded more attention. Tuoc emphasises that the VSRC is concerned with basic science, but that this will have a practical application in the future. Some biologists in his Institute are engaged on plant breeding programmes with rice seeds obtained from the International Rice Research Institute in the Philippines; their aim is to produce a crop with a higher nitrogen content and to find a rice variety that will withstand a colder environment. Others are studying diseases of rice and tobacco—two of Vietnam's most important crops. Some of the research in vitamins in progress will include a compilation of these nutrients in the country's foodstuffs. Zoologists are similarly engaged on a programme of cataloguing Vietnam's fauna. The two rooms allocated to them in the Institute of Biology are filled with animal specimens destined eventually



Zoological specimens await attention

for a museum of natural history.

Of the four institutes in the VSRC, physics is by far the best equipped. The physicists have some real successes to their credit, and are perhaps the most confident of Vietnam's scientists. Professor Nguyen Van Hieu is a vice-chairman and in charge of the Institute of Physics. Open, warm and very forthcoming, Hieu is a perfect ambassador for Vietnamese science. Hieu is a high energy physicist and a professor at the Dubna high energy physics institute near Moscow. His institute has strong ties with Dubna and some Vietnamese physicists are currently engaged there on 1 to 3 year contracts. Many of the physicists—like most of the other scientists in the centre—are either trained in the Soviet Union or Eastern Europe; some studied in France and Germany.

The real achievement of Hieu's institute is the completion of a five-year programme to establish Vietnam's electronics industry. With many patents and raw materials bought from the West the physicists now produce their own transistors. It has proved to be an expensive undertaking, but well worth the financial support provided by the grants from industry. Vietnam's technologists have also worked on the electronics programme for its duration and in preparation for the transition of the work from pilot stage to full scale production. 1978 is destined to be the year of the first Vietnamese radio components.

To offset the cost of the transistors the physicists—in co-operation with the earth scientists—are actively studying the properties of Vietnam's natural resources. They hope to find the necessary raw materials to assist with

manufacture. In the past most of the research of the institute was of an applied nature; only recently has it been possible to do basic research. The six departments in the physics section cover a variety of topics. In theoretical physics, there are quantum field theorists, and solid-state theorists. In other departments some study semiconductor physics; some optics—for prism and lens manufacture to study the optical properties of semi-conductors and solid state materials—and quantum electronics; some work on technical physics for physical measurement; some on the physics of radiation; and some on crystallography.

The chemistry institute is headed by Dr Khoi Nguyen Huu. Vietnam's chemists are also engaged on a study of the country's natural resources. Their interest is centred on plant terpenes, steroids and alkaloids. In co-operation with botanists, plants used in traditional medicine are being grouped according to their chemical taxonomy. Eventually the active ingredients in the medicaments will be identified and synthesised. Certain stages of the process are feasible at the moment, but for positive fingerprinting all chemicals still have to be sent to foreign laboratories, a process the chemists find very time consuming.

The chemists are also studying the oil resources in Vietnam. The final aim of the programme is to assist industry to extract commercially important oils from anise, citronelle, cinammon, basilicum, dugenol and mint. With further departments for inorganic analysis in Hanoi, and others for oil chemistry and organic synthesis—mainly polymer chemistry—in Ho Chi Minh City the chemists have the necessary manpower for their work. Again the real problem is a chronic shortage of equipment and of available chemicals and solvents.

The VSRC is the best equipped of Vietnam's research institutions. Its programme is closely allied to the country's needs. But like all of Vietnam's research institutions it lacks many resources. Scientists, scientific institutions and governments in the West could do a great deal to assist the development of science in Vietnam. The aid which is currently given to Vietnamese scientists by Western donors is still far from adequate. Far more in the form of literature, equipment and exchange facilities for scientific co-operation is required.

When Vietnamese scientists are so obviously keen to make contact with their Western counterparts it surely is an opportunity to be grasped. A generous response from the West would be in order, for Vietnam's burden is one few would wish to carry. □

US establishes new directorate for applied research

In a renewed attempt to develop an effective mechanism linking scientific research to national priorities, the National Science Foundation (NSF) announced last week that it is to set up a directorate for applied science and research applications (ASRA).

The new directorate, with an initial annual budget of about \$60 million will replace the Research Applications Directorate, which has been responsible for the increasingly-criticised programme of Research Applied to National Needs (RANN) set up in 1971 in an attempt to bridge the gap between basic research and its applications.

The main difference between RANN and the new directorate is that whereas the former was primarily organised around individual problems, ASRA has been organised on more general principles.

Thus the RANN programme has at present five separate foci, the three most important being resources, environment, and the loosely-defined field of 'advanced productivity research and applications'. In contrast, ASRA will be divided into six functionally-defined units, including an office of problem analysis, a division of integrated basic research, a division of applied research, and a division of problem-focused research applications.

In addition, whereas RANN operated relatively independently of other research programmes financed by the NSF, ASRA will work closely with the foundation's three directorates for basic science: for example it will help to identify and subsequently stimulate basic research relevant to agreed goals.

In the US, the NSF was placed on this path by a 1969 amendment to its original act under which the foundation was authorised to "support, through other appropriate organisations, applied scientific research talent relevant to national problems involving the national interest".

In line with this amendment, and with President Richard Nixon's emphasis in a State of the Union message on harnessing the "discoveries of science in the service of man", the RANN programme was set up with responsibilities that included the identification of unrecognised research needs, and increasing the effective use of science and technology in dealing with national problems.

Despite stimulating a number of important research developments—for example into the use of geothermal and solar energy—the organisational basis

of RANN has in recent years encountered growing criticism, reflected in a gradual fall-off in its budget appropriation.

RANN faced the dilemma that if it defined research areas too narrowly, then many research proposals would not fit; but if the definitions were too broad, it was accused of encouraging a 'shotgun' approach to problem-solving without any clear description of research objectives.

Further criticism came from the scientific community, who objected to the apparent attempt to impose unreasonable goals on research efforts. In a highly critical report on RANN's research efforts in the applied social sciences, for example, the National Academy of Sciences claimed that these projects were "of highly variable quality and, in general, not impressive".

Although the Research Applications Directorate introduced a new structure for the RANN programme in 1976, unease remained, and in December that year a task force was set up under the chairmanship of Dr John Whinnery of the University of California, Berkeley, to 'review and advise the director on science applications across the Foundation'.

Dr Whinnery presented his report last summer, suggesting a number of alternative structures, from which the new directorate for applied science and research applications was selected by the NSF. The directorate, which comes into effect next month, is divided into six units as follows:

- The office of assistant director, which will carry out the policy-making, management, review and co-ordination functions of the directorate;
- The office of problem analysis, which will work with internal NSF and external groups and organisations in assessing problems for strategic and programmatic planning;
- The division of integrated basic research, a new section which will provide a direct link between ASRA and the basic research directorate of NSF by jointly identifying basic research related to significant national problems;
- The division of applied research, whose two sections—one dealing with applied social and behavioral sciences and the other with applied physical, mathematical and biological sciences—will support research proposals benefiting social, economic and technical problems and policy issues, as well as identifying and stimulating the growth of new technologies and processes

based on discoveries in various fields of science;

- The division of problem-focused research applications, which will support the application of scientific and technological capabilities to selected problems of society which are of critical national importance. (This division will initially contain four research programmes: earthquake hazard mitigation, chemical threats to man and the environment, biological alternatives for industrial feedstocks, and community water management); and

- The division of intergovernmental science and public technology, responsible for integrating science and technology into federal and state planning.

The head of the new directorate will be Dr Jack T. Sanderson, who took over the Research Applications Directorate last summer on the resignation of Dr Alfred J. Eggers, RANN's first director and now director of Lockheed's Palo Alto research laboratory.

Dr Sanderson claims that part of the new directorate has responsibility for "the most complicated part of the research spectrum" in contrast to the organisation of basic research, with its peer group system and its established paradigms. Linking research to national priorities is, he admits, an area in which the necessary mechanisms are "least well understood".

The stakes are high; no one doubts that there is a close relationship between basic research and technical development—but no one has yet come up with a totally convincing (and effective) way of pinpointing precisely how support for the former can stimulate the latter.

Will ASRA succeed where RANN seems to have failed? According to Dr Sanderson, the organisation of the new directorate is based on various models, each of which has already demonstrated a certain success, thus the integrated basic research division is modelled closely on the NSF's energy-related programme, instituted in response to the energy crisis of 1973. Under this programme NSF officials and research scientists together identified 40 priority research topics for which extra funding was subsequently made available. "Similarly the model for the problem-focused research applications division was developed after looking at the way applied research activities are carried out in government and private industry" Dr Sanderson says.

So far, the reaction to the new directorate from Capital Hill, from which some of the most vocal criticisms of

RANN were heard, has been a cautious welcome. But President Carter is known to be carrying out assessment of the effectiveness of the current organisation of research funding. A \$4 million programme providing basic support

for university research, known as "basic research stability grants", has already been "impounded" by the President from the NSF's 1978 budget prior to the outcome of this assessment. ASRA will therefore no doubt

receive close attention from both the House and the Senate during the hearings on the President's proposals for the science budget, due to take place in March.

David Dickson

Shcharanskii may soon be brought to trial —without a lawyer

THE US National Academy of Sciences has made an unprecedented demand for permission to send a legal observer to the forthcoming trial of Anatolii Shcharanskii, the Soviet cybernetician, who, until his arrest last March acted as spokesman for the unofficial 'Sunday Seminars' and was an active member of the Moscow Helsinki Monitoring Group.

Since his arrest, Shcharanskii has been held incommunicado, pending investigation on charges amounting to treason. The official 9-months investigation period expired in December, whereupon the investigating officers requested and were granted an extension of six months. Nevertheless, Shcharanskii's mother has now been told that she should find a lawyer for her son, not later than Friday, January 13, 1978—a somewhat ironic instruction since already at least 30 Moscow lawyers have refused to act in his defence.

According to a TASS statement of 22 December, the charge against Shcharanskii is that of giving assistance to a foreign State by systematically supplying his 'masters' with slanderous information about the Soviet Union, which was then actively used for ideological diversion against the Soviet Union, and supplying to the West information about Soviet enterprises and institutions, data which constituted official secrets. Already quasijudicial 'hearings' in Stockholm, New York, Paris and London, have presented a considerable bulk of evidence in rebuttal of these charges. While the publicity value of such moves may be considerable, the comment of *Literaturnaya Gazeta* that it is only a court which can determine Shcharanskii's innocence or guilt and that it is not for US lawyers to maintain law and order in the USSR does have a certain justification. The move of the NAS, in requesting the presence of an observer at the trial, is possibly proving more difficult to answer, since, although the hand-delivered letter was accepted by the Soviet Embassy in Washington, to date no answer has been received—a standard Soviet practice in dealing with embarrassing requests from abroad.

The NAS appeal was made by its



Anatolii Shcharanskii: held incommunicado

Committee on Human Rights, which was founded in 1976 at the requests of the grass-roots membership of the Academy. The Committee includes some 50 members of the Academy and in all, out of a membership of 1,200, over 350 members have done active work on behalf of dissident or imprisoned scientists. To date, their most notable success has been the release of the Argentinian Juan Carlos Gallardo, although they freely admit that the credit for this must go not to the NAS alone but to the whole human rights movement.

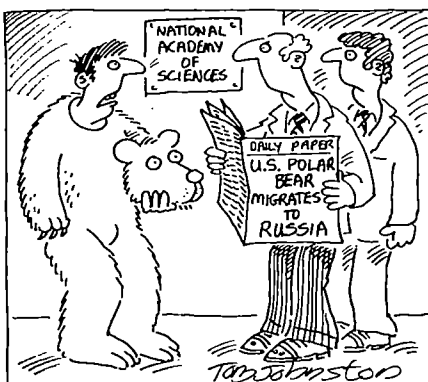
The Shcharanskii appeal noted the "widespread reaction . . . and grave concern" among Shcharanskii's colleagues in the USA and elsewhere, and suggested that further action against him would "severely damage scientific interactions with the USSR". This is not an idle threat—already the American Association of Computing Machinery has threatened to break off exchange relations with the Soviet Union over the Shcharanskii case. The Human Rights Committee of the NAS acts essentially as a mouthpiece of membership pressure. If there should be a widespread demand for a severance or suspension of such agreements

(and with more than a quarter of the membership actively involved in human rights activities, this is by no means impossible), the implications would be considerable. Although there exists a direct intergovernmental scientific exchange programme (established by the Nixon-Brezhnev talks of 1972), the exchange agreement between the Soviet and US Academies is a much older one (dating from 1959) and is both extensive and prestigious.

The NAS appeal is only one expression of the unprecedented concern which the Shcharanskii case has evoked. A number of factors are involved. One is, of course, the gravity of the charges. The choice of these charges, and suggestion of CIA involvement may in itself be a result of human rights action in the West. A few years ago, when intervention by such bodies as Amnesty International on behalf of dissidents became embarrassing, certain attempts were made to produce criminal charges so as to put the case outside Amnesty competence. Thus the refusenik physicist Viktor Pol'skii was accused of dangerous driving and manslaughter. The human rights activists promptly reformulated their terms of reference to include such cases, so that

from this point of view, the Shcharanskii case may be envisaged as the next move in a grim kind of human chess. Incidentally, the charge of disclosing state secrets is particularly ironic, since on graduation Shcharanskii carefully avoided taking a post where he might be exposed to classified information, which could hinder his chances of emigrating to Israel. Similarly, the Kiev physicist Vladimir Kislik, against whom, it is feared, similar charges may be in preparation is specifically accused of illegally sending abroad a scientific paper for publication. Not only is the information contained in it not secret; according to a western referee, the subject matter is so well known that he would not himself recommend publication.

Shcharanskii is somewhat of an exception among the refusniks, who, while awaiting their visas for Israel generally try to avoid dissident politics. The usual refusnik policy, as explained to *Nature* by Mark Azbel, former leader of the seminar is to avoid any confusion of the two issues, lest the



"Gee prof, there must be easier ways of getting in to observe the Shcharanskii trial"

would-be emigres be accused of subversion and the dissidents of Zionism. Whether such a distinction is possible is a moot point, since the Soviet authorities themselves seem bent on confusing it, by claiming, for example, that Sakharov is "really a Jew called Zuckermann". Shcharanskii, however, during his time in 'limbo' took an active interest in the Helsinki monitor-

ing group. Thus his arrest can be seen either as part of the renewed pressure on the Seminar, the 70 hard-core members of which are now being subjected to increasing surveillance including the use of cars with special listening devices, or as part of the campaign against the Helsinki monitoring groups two of which (in Ukraine and Armenia) had several members arrested over the (Western) Christmas holidays, when, it was presumably hoped, the news might pass unnoticed abroad.

From its very beginning, the Soviet human rights movement has been predominantly a movement of scientists, and this fact has undoubtedly contributed to an increasing concern about Western scientists with such problems. To date, except in the special case of psychiatry, where misuse of professional knowledge for political ends was involved, there has so far been no threat that protest abroad would lead to the severing of scientific relations. How far such sanctions will go in defence of Shcharanskii remains to be seen.

Vera Rich

Tory attacks energy gap forecasting—and the fast breeder

A BRITISH Conservative MP has attacked the hypothesis that there will be an 'energy gap' in the UK towards the end of this century, and questioned the economic sense of a commitment to a large programme of fast breeder reactors. The MP, Mr Nigel Forman, is the author of a Conservative Political Centre (CPC) pamphlet published this week titled *Towards a more conservative energy policy*. The document, while not a formal statement of Conservative Party policy, is according to a CPC spokesman "fairly close to the mainstream of Conservative thinking".

Mr Forman calls for emphasis on improving the efficiency of the production and distribution of energy, where 30% of primary energy is wasted, and proposes a flexible energy policy which operates on the demand as well as the supply of energy.

In a refreshing document which seems to verge on the radical rather than the conservative, the MP calls for more research on renewable resources, and for an approach to energy policy which will "liberate it from the requirements of the major vested interests". The conventional wisdom on renewable energy sources "seems to assume that they will make only a limited contribution to the energy supplies of the UK by the year 2000". However the main reason that the predictions are modest "is that until very recently

the R&D effort on nuclear energy, for example, exceeded that on all the renewable resources together by a factor of about 100".

Mr Forman brings his strongest criticism to bear on conventional energy forecasting and on the economics of the fast breeder programme. "It is when the experts begin to foretell the future that the trouble really begins" writes Forman. Undaunted by their predictions of an energy gap he argues that "what we are really being offered is little more than predictions of a change from a brief period of energy self-sufficiency in the 1980s and 1990s to a renewed period of energy import dependence—mainly for liquid hydrocarbons—at a time when the real price of energy is likely to be significantly higher than it is today. The threat, if there is one, is therefore to the prospect of continued exponential growth of final energy demand . . . it is not, nor need ever be, a threat to a society with improving rates of efficiency in fuel conversion and energy use".

An energy forecast is impossible, says Forman, without making prior political assumptions; and one of those assumptions ought to be that demand can be modified to meet supply.

On reprocessing and the proposed facility at Windscale, Mr Forman argues that "there simply will not be the necessary nuclear fuel throughput

from the Continent or elsewhere to justify such a large investment". Reprocessing, in other words, is uneconomic. The same is true of the fast breeder, says the MP, pointing out that the construction of around 20 breeder reactors of 1,300 MW each would cost a total of some £30 billion. "Simply to postulate such an enormous figure is to underline the foolishness of committing this nation to such a single-minded and excessive expenditure on a form of energy supply which we are unlikely to be able to afford and may not even need".

It may shock them, but with Prime Minister Jim Callaghan bearing down on Energy Minister Tony Benn to make him take a more pro-nuclear line it is beginning to appear that the best thing the Friends of the Earth could do would be to vote Tory.

Robert Walgate

Soviets look after their cosmonauts

THE purpose of the Soyuz-Salyut programme is not the carrying out of separate experiments, however unique. In a recent Tass statement, Flight Director Vadim Kravets announced that the ultimate aim is the establishment of a permanent scientific watch in orbit.

Accordingly, much is being done towards cosmonaut comfort and better working conditions, including the installation of a shower in Salyut 6 and the introduction of new semi-rigid space suits for extra-vehicular activity.

Since Salyut 4, the special computer-based Kaskad orientation system has been used to re-align the station as required by the programme of geo-physical, astro-physical, and solar observations, while the Delta autonomous navigation system has eliminated the exhausting and tedious work of orbital correction.

The possibility of natural disaster in orbit is considered minimal—pilot-cosmonaut Georgii Grechko has been quoted as saying that a direct hit by a meteor would be likely only once in two thousand years. Nevertheless, a Tass report of 24 December notes that a micrometeorite groove 1.5 mm deep has been observed on the glass surface of one of the Salyut 6 portholes. Grechko remains unruffled. □

First catch your bear . . .

AN unscheduled contribution to Soviet-US co-operation in the ecology of the Far North has been initiated by an Alaskan polar bear which, wearing a collar with a radio-transmitter attached, has set out across the Bering Strait to Siberia. When last observed, the bear appeared to be making for Wrangel Island, an area in which unfortunately, no major expedition is working at present. It has been agreed, however, that should the staff at the reserve base on Wrangel Island spot the bear, the collar and its transmitter will be returned to the Americans. □

\$17m for Salk institute

THE Salk Institute for Biological Studies is to set up a new "government services division" to carry out contract research for various government agencies.

This move has been made possible by the donation to the institute of the vaccine research and production facilities of a major pharmaceutical manufacturer, Richardson-Merrell, Inc. The value of the facilities has been assessed at \$17m.

The manufacturing portion of the facilities has already been sold. The institute will retain the research portion of the facilities, situated in Swiftwater, Pennsylvania, including 25 professional and technical personnel. □

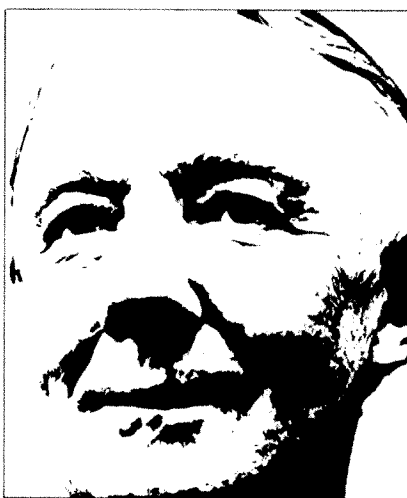
MAN, with his increasing numbers and his wasteful use of resources, may damage both his immediate environment and the globe as a whole. Most of us wish to try to make the world a better place to live in, or at least to prevent further damage to its natural amenities. However, those concerned have different approaches to environmental problems, and it is perhaps worthwhile trying to decide which is the most effective.

Most publicity is given by the media to the extreme 'doomwatchers', who think that the only way to produce results is to frighten mankind into mending his ways. This was the technique of the Victorian hell-fire preacher. Today some environmentalists seem almost to exult in the prospect of impending disaster, as did the earlier preacher at the probability that his listeners would suffer the tortures of eternal damnation.

These extremists are unwilling to agree that there has ever been any improvement—they may even believe that this is the case. They are scornful of those with a different approach, and have their own vocabulary of insults. Those who express even the most qualified optimism are "complacent", and when this optimistic picture is supported by evidence that at least one type of damage had been controlled this is described as "simplistic". Even when it is impossible to deny that there has been some improvement they think up cryptic and subtle dangers from which we are suffering without being aware of their presence.

On the other hand we have those who take a more moderate line. They know that resources are limited, and they wish to make sure that these are used effectively. They wish to identify and quantify all dangers, so as not to waste their efforts on trivial and unimportant factors. As this group tends

Postponing doomsday



KENNETH MELLANBY

to lack glamour, it is seldom seen on television.

One organisation which has a reputation for moderation is the National Society for Clean Air (NSCA). I attended their annual conference at Harrogate recently. The members of this society are mostly recruited from the local authorities which are responsible for environmental protection, together with government and other scientists expert in different fields. The atmosphere at their conference was one of hope, not of doom. Most of the papers recorded growing improvements in the air quality of urban and industrial areas in Britain, and few spectacular dangers of any magnitude were reported.

The relative unimportance of the existing levels of substances like carbon monoxide and lead, which would be disastrous at higher concentrations, was discussed. However,

the conditions in areas with laggard local authorities, where smoke control was not enforced, were fully described, and those responsible were duly castigated. The members of NSCA had no doubt that their own efforts had contributed substantially to the real improvements which they reported. Some listeners complained that the Society was unduly complacent, and that it should be more militant even when existing pollution levels were apparently harmless and were, anyhow, decreasing, but these views received little support.

It will be clear that my sympathies are with NSCA. They get things done. Extremists will say that this is, at least in part, because doomwatch publicity has affected public opinion, and made it ready to respond to moderate views which might otherwise be ignored. I am doubtful. In the late nineteen sixties we were told that growing air pollution would make the major cities of Europe and America uninhabitable before the end of the century. Most and most people can recognise that things have got better and not worse. The Victorian preacher did not stamp out sin; many of his congregation did not really believe in the hell he promised them. The doom-besotted environmentalist is in an even worse position, for there is concrete evidence that his particular hell, if it exists, is getting further away as the moderates get on with the job of environmental improvement.

However, the world is still far from perfect. The extremists are not always wrong, and though their efforts at frightening the general public may be counterproductive, the experts would be wise to pay at least some attention to their views, for the cryptic dangers they stress have, at least in some few cases, been shown to be real.

correspondence

In support of a boycott

SIR,—We read with great interest the letter by Richard Peto and Richard Doll (1 December, page 384) concerning the congress of the International Union Against Cancer which is scheduled to take place in Buenos Aires, Argentina October 1978 and the advisability of conducting a boycott in view of the violations of human rights in that country. It is stated that "advice and comments from other scientists, especially those who, unlike ourselves, have worked under repressive regimes, would be timely, both about the general advisability or inadvisability of such boycotts" in a country where there is conclusive evidence of widespread repression, torture and systematic violations of human rights.

The Association Solidarité Franco Argentine (a non-profit making, humanitarian organisation based in France) has contacted many Argentinian scientists regarding this problem. Although they asked not to be identified by name for fear of the consequences to their families still living in Argentina, the general opinion is that whatever measures are taken to show concern for the fate of the many scientists that have simply "disappeared" at the hands of the military government, there is one thing which should not be permitted: that the Argentinian government should use the success of the congress to claim to the world that the scientific community condones its present policies, by being present at the meeting and keeping silent about the plight of Argentinian scientists.

Unfortunately this is what seems to be happening in Argentina right now. A recent article from the strictly censored local press says that in spite of the efforts of some scientists, who pretend to be motivated for humanitarian reasons but in fact echo slogans of international terrorism, the congress will be a success. To prove this, the article mentions that only 500 participants had registered for the previous Florence meeting, compared to the 3,000 for the Buenos Aires event. Dr Humberto Veronessi, President of the International Union Against Cancer, is quoted as having said "I believe that to choose Buenos Aires for this meeting is one of the most

intelligent actions taken by the Union". This type of article suggests that the Argentinian generals have already invested high hopes in a "business as usual" congress as a measure of international acquiescence.

Among the many well known scientists to have publicly protested against the terrorising policies of the military Junta are Nobel prize winners Louis Néel, Alfred Kastler, Tsung Dao Lee and Hans Bethe (*New York Times*, 27 November, 1977). Concerned scientists attending the Buenos Aires meeting can take similar action by openly voicing their concern about the many Argentinian scientists who have vanished. This could be done by organising a petition to be read at the meeting or a press conference to be held in the presence of foreign journalists.

What has happened to Drs Federico Alvarez Rojas, Antonio Misetich, Gabriela Carabeli, Eduardo Pasquini, Julia Huarque, Federico Ludden, Manuel Tarchitsky and Juan Carlos Gallardo, all of them physicists, and the many others kidnapped, in many cases, with their families? Why aren't the numerous concentration camps open to Red Cross personnel? There is every reason to suspect that the worst is happening, just as it did in Nazi Germany, where similar questions puzzled many scientists, and massacres were taking place not far from the place where scientific meetings were being held.

Exiled Argentinian scientists beg the rest of the scientific community to stand up, whether by boycotts or in other ways. "Anti-science" sentiments will certainly not be aggravated by a public stance against torture and death. Many lives may instead be spared.

Yours faithfully,

GUY TASSART

*Association Solidarité
Franco Argentine,
Grenoble, France*

Soft energy paths

SIR,—Peter Chapman is right to say (10 November, page 128) that my US calculations in *Soft Energy Paths: Toward a Durable Peace* should be redone for the UK—as several colleagues are now attempting. But in suggesting how my US numbers "fail on a num-

ber of technical issues" he may mislead UK readers unfamiliar with transatlantic differences.

First, he says my 0.55 capacity factor for US pressurised-water reactors (PWRs) is "unrealistically low", "absurd", and "silly", and so it must seem to people used to British gas-cooled reactors which are far more reliable (though allegedly more costly). Yet my citations show that the US empirical average through 1976 is 0.61 (0.59 for all light-water reactors) and falling as less reliable larger plants come on line; that the officially projected lifetime average is 0.57; and that the 10-year levelised average expected for new big PWRs on the basis of exhaustive statistical analysis of existing units is 0.49. Thus my assumed 0.55 is arguable either way but hardly out of court under US conditions. (European units tend to do better, Japanese ones worse.)

Second, in my analogy suggesting that if we could mass-produce power stations the way we do cars they would cost an order of magnitude less than they do, but we can't because they're too big, I did take account of generator cost and of equivalent engine lifetime. (I also noted many important economies of small scale—related to reliability, reserve margin, short lead times, etc—which are not "very dubious" nor even empirically controversial. For a taxonomy of scale issues, see my "Soft Energy Technologies" in the 1978 *Annual Review of Energy*.)

Third, the \$100/(m²+m³) solar installed price Dr Chapman quotes is for the mid-1980s (in 1976 dollars), not now; my late-1970s estimate of \$150 is broadly consistent with his. My ca 1985 price estimate is based on empirical prices now obtainable by careful shopping in a fairly mature solar market (such as California, with due adjustments for different climatic needs). Current UK prices in an infant market with little volume or diversity are understandably higher but do not indicate what could be achieved. I do not see why the "cost of storage in the UK" should be £300/m³ or even £40/m³, as I cited empirical installed US prices, for modular underground tanks of tongue-and-groove concrete slabs, equivalent to £12-20/m³ for sizes of order 10–10² m³, and several even cheaper methods are available. Perhaps they are

not in use in the UK, but that is not my fault. Further, solar heat's marginal-cost advantage is not fragile but robust: even with collectors costing twice Dr Chapman's assumed £50/m², neighbourhood-scale seasonal-storage solar space heating in the UK should compete with any long-run marginal source and probably with OPEC oil too (see the *Ann. Rev. En.* article).

Fourth, my analysis assumed neither large wind machines nor growing special biomass crops (rather, it assumed the conversion of present farm and forestry residues requiring no additional land); and I did not ignore, but repeatedly emphasised, the economic argument for matching energy quality to end-use needs. One of the reasons for persistent official commitments to a hard energy path is the prevalence of asymmetric cost comparisons: governments compare the costs of various types of big power stations and synthetic-fuel plants with each other, then compare the costs of soft technologies not with their hard-technology competitors but with the historically cheap fossil fuels that all are meant to replace. This makes some soft technologies fail a test which hard ones would fail by a far wider margin. So long as such chicanery goes unremarked, economically and politically disastrous energy policies will continue to prevail over common sense.

Yours faithfully,

AMORY B. LOVINS

*Friends of the Earth,
London, UK*

Gene inquiry is timely

SIR,—Your editorial (8 December, page 461) criticised the decision of the House of Commons Select Committee on Science and Technology to set up a subcommittee on genetic engineering. It did so in remarkably complacent terms which seem to us to pass all too lightly over the problems which remain unexamined and unresolved in this area and to exaggerate the extent to which public debate has occurred. In our view it is not true to say there has been exhaustive scrutiny or debate in the UK of the issues involved. The "general feeling" of scientists involved in the field may be that the hazards have been overplayed, but this attitude ignores other issues which concern the public.

Many of the hazards involved in genetic engineering require much wider examination. The analysis so far has been far from comprehensive, and the actions taken incomplete. For example, recommendations made by the Ashby Committee three years ago, such as the institution of epidemiological surveys

of workers in communities where these experiments are undertaken, have not yet been implemented. And the body established to regulate work in this field (the Genetic Manipulation Advisory Group) has few powers, relies on voluntary cooperation and is already experiencing problems in dealing with confidentiality of industrial information.

The examination of this area by the select committee could achieve much. It could help bring about a much wider appreciation of the far-reaching issues involved. It could also provide a valuable independent assessment of the policy-making procedures being created in this area which are currently screened from the public gaze by the protection of the Official Secrets Act. Now is the time for such an examination, before the problems of industrial exploitation are upon us. This is an urgent matter which requires as thorough an analysis as that at last being given to nuclear power.

We applaud the initiative taken by the select committee and feel sure that it will take the opportunity to take note of the wide range of views on this topic.

Yours faithfully,

BRIAN CUMMINS

MARK PINEY

JON TURNEY

NEIL WALDEN

EDWARD YOXEN

*British Society for Social
Responsibility in Science,
London*

What happened at Heimaey?

SIR,—In his search for a deontological code for volcanology, Haroun Tazieff (8 September, page 96) has elected not to practise his own preaching and indeed based some of his own arguments "on deliberately false data". We are astounded by his inclusion of the water-chilling of the Heimaey lava in 1973 in his tabulation of erroneous volcanological diagnoses, and his account of countermeasures taken by Icelanders as defence against lava flows on Heimaey indicate either lack of familiarity with relevant literature or wrong interpretation of actual facts.

In an attempt to prevent westward advance of the Heimaey lava over the town and towards the harbour, earth dams were bulldozed in late January and early February 1973. Experiments with chilling of lava-fronts by water-pumping started on 6 February and while lava advance could not be prevented, local slowing-down and diversion was achieved. Thus chilling of lava in this way is believed to have saved electric power-line installations for a while and diverted lava from the harbour wall on

6 March. Subsequently, pumps with total capacity of 1,000 litres per sec were employed, feeding a network of 20 cm diameter flexible plastic tubing system from the harbour to the lava fronts which were threatening further destruction of the town. This large-scale operation resulted in doubling of height of some lava fronts (Th. Einarsson, *The Heimaey Eruption*, Heimskringla, Reykjavik (1974)) as production of clinker and blocky rubble was increased on the lava surface. This increasing clinker accumulation rate seems to have decelerated or halted advance of the lava in certain areas.

Our knowledge of the mechanics of lava movement is still rudimentary. Recent theories, such as that of Hulme (*Geophys. Journ. Roy. Astr. Soc.* (1974)), make it clear that the strength of the flow front and channel levees are of great importance in controlling lava shape. As lava levees or flow fronts are made stronger and thicker, lava builds up behind these natural barriers. Lava will clearly attempt to break out or advance at the weakest front. By preferentially strengthening a levee or flow front by such a technique as water-cooling it seems probable that the lava will prefer to advance elsewhere. Levees or flow fronts are only minor parts of the total lava at any time, but increase in the strength of these areas may be highly effective in diverting lava. By choosing a strategic zone such as a levee, cooling need only be concentrated on a small part of the flow. Evidence from the Heimaey experiment suggests that the uncooled flow front ranged 10 m to 15 m in height, whereas the flow front treated by water pumping ranged from 20 m to 30 m and possibly as much as 40 m.

Finally there is a requirement to substantially improve our understanding of lava flow mechanisms. This is an area of research that illustrates the importance of volcanology turning from a qualitative to a quantitative science. In this way some of the noticeable subjectivity in judging volcanic phenomena, which is amply illustrated in Dr Tazieff's note, can be replaced by informed opinion, based on detailed understanding of the physics and chemistry of volcanic processes.

The determination of the people in Heimaey in fighting the advancing lava was not daunted by defeatist utterances of some sceptics at the time of the eruption. We hope that readers of the otherwise useful note by Haroun Tazieff will likewise dismiss his pessimism about the usefulness of water-cooling in diverting lava flows.

Yours faithfully,

HARALDUR SIGURDSSON

STEPHEN SPARKS

*University of Rhode Island,
Kingston, USA*

news and views

Tangshan 1976: a case history in earthquake prediction

from Cinna Lomnitz and Larissa Lomnitz

Was the Tangshan earthquake of 1976, an event of Richter magnitude 7.8, predictable in the present state of earthquake forecasting in China? We shall attempt to show that it was not; in the absence of well-defined procedures of earthquake forecasting outside China our case rests on the detailed information obtained on two other large events, the Lungling and Yenyuen earthquakes, both accurately predicted by Chinese seismologists in 1976. A 10-day briefing in China has been of the greatest importance in making this report.

Chinese earthquake forecasting is a structured process of four stages: long-range, middle-range, short-range and immediate predictions. In the case of Tangshan a long-range prediction was issued in 1966, implicit in a directive of the Central Committee of the Communist Party, requesting seismologists to pay particular attention to North China and specifically to the Peking-Tientsin-Haicheng triangle. This directive was issued after the 1966 Hsingtai earthquake.

After the successful prediction of the Haicheng earthquake of 1975 it was generally expected that the long-term strains accumulated in the Peking-Tientsin area had been released (Fig. 1). This was a reasonable assumption because the region of North China is only moderately active. Yet there has been clustering of large events in time: for example, two shocks of magnitude 8 occurred in 1679 and 1695. No major earthquakes had previously been recorded in the immediate vicinity of Tangshan; the nearest events were a coastal shock ($M = 6$) in 1568, and an earthquake in Langcheng County ($M = 6\frac{1}{2}$) in 1624.

The earthquake occurred at 03:42:56 (Peking time) on 28 July, 1976. The location of the epicentre was 39.43°N 118.15°E , with a focal depth of 12 km. Twelve aftershocks exceeded mag-

nitude 6; the largest ($M = 7.1$) occurred on 28 July in Langcheng County—about 50 km to the northeast of the main shock.

Tangshan is in the centre of a rhomboidal area outlined by major crustal faults. The Tangshan fault proper is at least of Palaeozoic age; it is 40 km long, and runs along the top of a narrow uplifted block of Ordovician age. From geological and geophysical evidence it was concluded that the earthquake had been caused by a regional compressive stress from $\text{N } 86^{\circ}\text{E}$.

The main fault broke along an 8 km trace which went through the middle of the city of Tangshan. Right-lateral displacements of up to 1.58 m were observed. The vertical component of displacement of the east block was down towards the northern end of the fault break, and up towards the south. The trend of the fault trace was about $\text{N } 40^{\circ}\text{E}$; however, the fault trace of the main aftershock was nearly north-south. The displacement of this fault

was left-lateral. The aftershocks fell into two groups: the areal distribution and focal mechanisms for each group were consistent with each of the two fault traces. Hence the Tangshan earthquake may be described as a twin event.

The two earthquakes to be used as comparison were also twin events. The Lungling earthquakes (Southern Yunnan, 29 May, 1976; magnitudes 7.5 and 7.6) were successfully predicted; the immediate prediction was made by an amateur group at Lungling about 20 min before the first main shock. The Yenyuen earthquakes (Yunnan-Szechuan border, 7 November, 1976; magnitude 6.9, and 13 December, 1976; magnitude 6.8) were also predicted but the immediate forecasts were issued 3 days in advance. No lives were lost in any of these quakes.

Forerunners

At least 10 different kinds of phenomenon are monitored for purposes of earthquake prediction in China. They include observations of fault creep, regional and local tilt, gravity, shallow earth strains, direct current resistivity, groundwater fluctuations and changes in spring flow, telluric currents, geomagnetic observations, geochemical observations (particularly of radon in groundwater), changes in the velocity of seismic waves, changes in the distribution of small earthquakes in space, magnitude or time and observations of foreshocks. All these are quantitative forerunners. There are also a few qualitative forerunners, such as changes in animal behaviour and in the groundwater regime: these are termed 'macro-forerunners'.

The following forerunners were identified in connection with the Tangshan earthquake.

- Fault creep. Two stations located at 180 and 200 km from the epicentre, on the Babaashan Fault, recorded a 25-fold increase in the creep rate after May 1975. The fault became locked

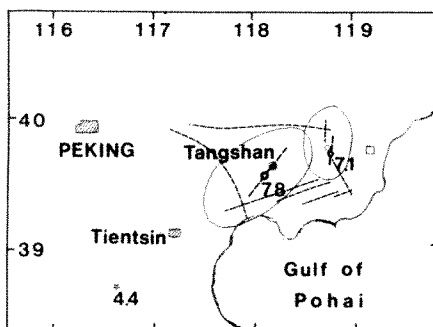


Fig. 1 Map of North China indicating the epicentre of the Tangshan earthquake (Peking magnitude = 7.8), and the second main event near Langcheng ($M = 7.1$). The ellipses represent the aftershock regions. The epicentre of the 'warning shock' near Tacheng ($M = 4.4$) is shown near the south edge of the map. Crustal faults outlining the 'Tangshan Quadrilateral' are indicated by broken lines; the two faults associated with the major events are represented by thick lines. The square symbol indicates Changli, a station which recorded a resistivity anomaly on 23 July, 1976.

between October 1975 and April 1976, then accelerated again to 33 times the average creep rate. About 20 days before the earthquake the direction of fault motion was suddenly reversed—unfortunately, at a time of heavy rainfall. (The effect of rainfall on the motion of the Babaashan Fault is not fully understood, but it is known to be a major effect).

● **Gravity.** Between July 1974 and July 1976 there was an increase of 100 μ gal in the Bouguer anomaly over the Tangshan quadrilateral. This was considered to be barely significant because the mean error was estimated at 40 μ gal.

● **Strain measurements.** Between May 1975 and July 1976 the quartz-rod strainmeter at Dahuidyan Observatory, across the Babaashan Fault (180 km from the epicentre), recorded a shortening of 0.3 mm, indicating regional compression. Piezoelectric strainmeters buried in pipes at depths of 3–10 m detected a sudden strain anomaly immediately before the earthquake. Dilatations and compressions were distributed in quadrant fashion about the epicentre.

● **Direct current resistivity.** Changli station, 80 km east of Tangshan, recorded a large decrease in resistivity beginning on 23 July. Between 30% to 40% of resistivity stations in North China showed some anomaly, but none was as clear as the one at Changli. The anomalies were widely scattered over distances of up to 300 km from the epicentre.

● **Fluctuations in wells.** Taking 23 July as a pivotal date, there was a marked increase in the reports of anomalies in wells. Only 59 such reports came in during the 5-day period preceding 23 July, but the number increased to 665 in the following 5 days.

● **Telluric currents.** Measurements of telluric current were not found to be particularly useful as the records were erratic. At Hsingchuang station, near Tientsin, a large telluric peak was recorded 13 min after the main shock, possibly due to some seismic coupling effect.

● **Geomagnetism.** No geomagnetic anomalies were mentioned at any time.

● **Radon.** Most Rd values had been increasing in North China since 1968. After February 1974 there were seemingly erratic fluctuations; after the Tangshan earthquake the Rd content has been dropping nearly everywhere. But there were no specific anomalies in the Tangshan area.

● **Small-scale seismicity.** There was no clear decrease in the minor seismicity after the 1975 Haicheng earthquake, as might have been expected if the regional strains had been released. A seismic gap had developed in the 'Tangshan quadrilateral' since about

Table 1 Short-range to immediate prediction

Time	Tangshan	Lungling	Yenyuen
<i>E</i> , 9 days	Resistivity drops at Changli Station	Increasing anomalies of Rd, magnetics, wells, hot springs, others	First foreshock, $M = 4.3$. Tsuli well dries up.
<i>E</i> , 3 days	Increase in well reports	Tsuli well dries up	Short-range forecast: E , 2 d, $M = 6-6.5$
<i>E</i> , 2 days			Second foreshock, $M = 4.0$. Tsuli well recovers
<i>E</i> , 1 day		Short-range forecast: E , 2 d, $M = 5-6$ Tsushun tilt base shows anomaly Large magnetic anomaly; animal behaviour; wells Foreshock, $M = 5.2$ More foreshocks Immediate forecast: Evacuation	Immediate forecast: Evacuation
<i>E</i> , 10 hour			Tzushun tilt base shows anomaly
<i>E</i> , 25 min			
<i>E</i> , 0	Earthquake		

1967; during 1975 this gap was outlined more clearly as only 14 shocks of magnitude 2.5 or greater occurred within the gap. The minor seismicity tended to migrate towards Tangshan: by 1976 many small shocks were occurring close to the edge of the gap.

Within the Tangshan quadrilateral the b -value decreased from 1.0 to about 0.35 between 1967 to 22 April, 1976. On this date an earthquake of magnitude 4.4 occurred in Tacheng (Hopei), near the southern edge of the gap. Afterwards the b -value recovered to about 0.6 just before the Tangshan earthquake.

The v_p/v_s anomaly about Tangshan followed a similar pattern. However, these anomalies were apparently detected after the earthquake, not before.

● **Foreshocks.** The seismographic record of the Tangshan station was recovered from the ruins: it showed only a few local shocks of magnitude less than 2 during the hours preceding the earthquake. This is conclusive evidence of the absence of foreshocks.

A comparative time history of forerunners of the Tangshan, Lungling and Yenyuen earthquake is shown in Fig. 2. At first sight no significant difference in the patterns emerges—up to the intermediate-range prediction. An intermediate-range prediction was indeed made for all three events. The magnitudes of all three earthquakes were underestimated. The differences appear at the short-range prediction stage. The comparative table above clarifies this point (E =time before the earthquake).

Notice that geophysical reversals (marked with an arrow in Fig. 2) were conspicuously absent before the Tangshan earthquake. The two exceptions (b -values and fault creep on the Babaashan Fault) were detected after

the earthquake, and neither could have been used to pinpoint the epicentral area. The Tacheng 'warning shock' of magnitude 4.4 occurred at a considerable distance from Tangshan. In contrast, foreshocks did occur both at Lungling and at Yenyuen, thus enabling local seismologists to close in on the epicentre weeks in advance of the earthquake. The onset of major foreshock activity, combined with sudden reversals of local tilt, magnetic field or fluctuations in ground water, will usually trigger an immediate prediction followed by evacuation.

A few words about the philosophy of earthquake prediction in China may be in order. The 1966 directive, usually attributed to Chou En-lai, outlined a five-point policy of earthquake prediction research: (1) give priority to prevention; (2) join the efforts of specialists and amateur workers; (3) combine indigenous and foreign methods; (4) work through the masses, (5) under the leadership of the Party. This policy has led to a distinctive scientific approach to seismological work. Initially many Chinese seismologists were reluctant to abandon deductive procedures based on the concatenation of hypotheses rigorously tested under controlled field situations. The key role of the masses was not recognised. Yet the observatories manned by specialists were too far-flung to be of practical use in detecting forerunners. Thousands of 'mass stations', built and operated by amateur observers, were needed to fill the large gaps between professional stations. Even those Chinese colleagues who remain moderately sceptical about earthquake forecasting methods now readily concede that the close collaboration between professional scientists and amateur observers represents a key element in achieving successful predictions.

Chinese seismologists do not claim any special knowledge concerning the cause and mechanism of earthquakes. After the successful prediction of the 1975 Haicheng earthquake, an official of the State Seismological Bureau bluntly warned: "There will be errors and disappointments, as well as unavoidable mistakes in reporting or failing to report individual forecasts . . . Earthquake prediction is a major complex scientific problem which overlaps many disciplines and which can hardly be expected to be solved at short notice . . . We have a long way to go. It will take time and much hard work". This note of caution, sounded 7 months before Tangshan, was by no means an accidental or isolated statement. Serious failures of prediction have occurred before the Tangshan disaster and will occur again. However, as long as the mechanism of earthquake generation is as imperfectly understood as it is today, the Chinese approach to prediction represents probably the most effective tool for controlling earthquake hazards in seismically active regions.

We are grateful to the State Seismological Bureau in Peking and to Chinese seismologists, for permission to reproduce the results of their investigations. Special thanks are due to Gao Wen-hsue of the State Seismological Bureau, Jin Hsue-shen and Li Yu-so of the Hopei Province Seismological Bureau, Ko Shen-min of the Institute of Geology, Ku Kung-hsu, Fu Cheng-yi, Sung Liang-yu, Chu Chun-zhen and

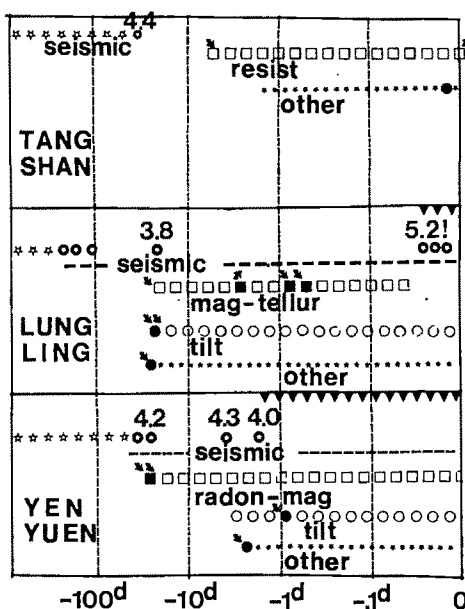


Fig. 2 Forerunners of the Tangshan, Lungling and Yen-yuen earthquakes, as referred to the time of the first main shock. A sawtooth pattern at the top indicates evacuation following an immediate prediction. Note that foreshocks serve to pinpoint an epicentral area, which is helpful in interpreting other anomalies. Intermediate prediction in all three cases occurred 15–150 days before the shock. Arrows, reversals; filled symbols, anomalies detected as significant.

Shi Jen-liang of the Institute of Geophysics, and Sung Chang-jiang, Chu Cheng-nan, Wang Feng-chi and Lin Tsun-yun of the Yunnan Province Seismological Bureau. □

Reabsorption of digestive enzymes: playing with poison

from Jared M. Diamond

In the course of digestion, the intestine and associated glands secrete quantities of solutes and water so large that the body could ill afford to lose such quantities in the faeces. Hence it is no surprise that distal parts of the intestine reabsorb most of these substances. Physiologists have studied in detail how, in a cycle termed the enterohepatic circulation, bile salts secreted by the liver are reabsorbed by the ileum and return by way of the bloodstream to the liver; and how salts and water secreted by salivary glands, stomach, liver, pancreas, and intestinal glands are reabsorbed between the duodenum and colon. Nevertheless, until recently there was no awareness of a cor-

responding circulation of the enzymes which the pancreas secretes into the intestine to split proteins, fats, and starches. Indeed, when evidence for such an enteropancreatic circulation was reported in 1975, it aroused 'interest but alarm' (on the part of Beynon and Kay, *Nature* 260, 78; 1976).

Reasons for the alarm were several. First, enteropancreatic circulation would require protein absorption by the intestine. While this has been demonstrated in some studies, much more attention has been paid to protein hydrolysis to dipeptides and amino acids in the gut lumen and subsequent absorption of the dipeptides and amino acids. Second, proteolytic enzymes and lipases might be expected to wreak havoc in the bloodstream, as illustrated

by their potency as the active ingredients of many venoms. Finally, there was no evidence for protein uptake from the bloodstream by pancreatic cells.

The missing evidence, and cause of the alarm, was provided by Rothman and colleagues (University of California Medical Center, San Francisco), who have added to the evidence (and to the alarm) in recent publications (*Science* 189, 472; 1975; *Nature* 257, 607; 1975; *Lancet*, 4 September, 494; 1976; *A. Rev. Physiol.* 39, 373; 1977; *Proc. natn. Acad. Sci. U.S.A.* 74, 4068; 1977). Their chain of evidence is as follows.

- Fifteen minutes after instillation into the gut of radioactive chymotrypsinogen, the inactive form of the proteolytic enzyme chymotrypsin, the radio-label appears in the pancreatic juice in a form comigrating electrophoretically with chymotrypsinogen.

- If pancreatic juice is diverted from entering the gut lumen and is instead drained off by a surgical fistula, protein secretion by the pancreas decreases 80–90%. Protein secretion is restored if pancreatic juice is returned to the gut or bloodstream. The calculated efficiency of enzyme recirculation, 80–90%, is comparable to that of the enterohepatic circulation of bile salts.

- The starch-splitting enzyme amylase is absorbed with its activity intact by a mechanism dependent on aerobic metabolism in the ileum. Absorption of a control high-molecular-weight substance, the physiologically inert polysaccharide inulin, is negligible under the same conditions. Similarly, Papp, Feher, Folly, and Horvath (Hungarian Academy of Sciences, Budapest) have reported duodenal absorption of pancreatic lipase intact (*Abstract, 1977 European Pancreatic Club Meeting*, 23).

- Radiolabelled chymotrypsinogen and amylase in the solution bathing or perfusing the isolated pancreas are taken up by the pancreatic cells and appear in pancreatic juice. The secretion rate of radiolabelled amylase in juice is increased by pancreozymin, the hormone that stimulates amylase secretion physiologically. That these phenomena are not due to breakdown and reincorporation of the radiolabel is shown by the negligible label in juice if the radiolabelled protein presented is albumin; by the absence of label in secreted amylase if the label presented is chymotrypsinogen; and by inhibition of labelled chymotrypsinogen secretion by an excess of unlabelled chymotrypsinogen, as expected for a saturable transport mechanism.

- There is a significant flux of amylase from pancreatic cells to an external bathing solution as well as into the pancreatic duct. Controls argue against

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this being attributable to a duct-to-bath shunt or cell damage. Thus, there is a two-way flux of amylase across the pancreatic cell membrane facing the bloodstream.

● Instillation of greater than normal quantities of pancreatic juice into the intestine of rabbits leads to 50% mortality—the only plausible toxic agents in pancreatic juice being the pancreatic enzymes themselves.

Mysteries remain. For efficient use of the enzymes in the gut, intestinal absorption should occur distally in the ileum as for bile salts, yet Papp *et al.* reported proximal (duodenal) absorption. Injection of proteases and lipases into a victim's bloodstream by poisonous snakes has spectacular consequences; why are the consequences of the postulated enteropancreatic circulation not equally spectacular? Are only the inactive forms of the enzymes absorbed by the intestine? If so, are the active forms lost in the faeces, and how can this be reconciled with 80–90% efficiency of recirculation? Does the intestine itself inactivate the enzymes while absorbing them? Both alarm and interest may be expected from physiologists until these questions are answered. □

Liposomes in biology and medicine

from G. Gregoriadis

A conference on Liposomes and their Uses in Biology and Medicine was held under the auspices of the NY Academy of Sciences on 14–16 September, 1977. It was chaired by D. Papahadjopoulos, Roswell Park Memorial Institute, Buffalo.

LIPOSOMES have been developed over the past 10 years or so both as model systems for the study of cell membranes and, as possible, vehicles for directing drugs to specific targets in the body. These two apparently unrelated aspects of the liposome were brought together at the conference.

The plethora of data on the physical properties of liposomes in their role as model membranes is now proving essential to the study of their interaction with cell membranes in their role as drug delivery vehicles. The opening lectures therefore—on the molecular organisation, lipid mobility, thermotropic and other properties of liposomes—were extremely relevant to their therapeutic applications. Fluidity

of liposomal membranes, for instance, which determines many of their structural and biological functions (interaction with cells for example) can be modulated by the incorporation of various molecules, by the hydrogenation of unsaturated phospholipid components (D. Chapman, University of London) or by Ca^{2+} which induces phase separation and subsequent fusion between liposomes (D. Papahadjopoulos, Roswell Park Memorial Institute, Buffalo).

Evidence of endocytosis as a mode of liposome–cell interaction was supplemented by alternative mechanisms such as fusion with mammalian cells (G. Poste, Roswell Park Memorial Institute), bacteria (M. J. Osborn, University of Connecticut Health Center) or viruses (A. M. Haywood, University of Rochester), as well as adsorption (R. E. Pagano, Carnegie Institution of Washington, Baltimore). However, such mechanisms, which relate to liposomal membrane fluidity and to cell or viral membrane components, may be less relevant *in vivo*. For instance, fusion has been seen in the absence of serum, components of which (α_2 -macroglobulin for example) adhering to liposomes could promote phagocytosis. Nonetheless, the possibility that mechanisms could be selectively induced to enable the access of liposomal drugs to a particular target organelle was raised by C. de Duve (International Institute of Cellular and Molecular Pathology, Brussels) who in this way introduced the conference to the exciting prospects of medical applications. These ranged from the treatment of tumours, enzyme and hormone deficiencies, metal storage diseases and arthritis to immunopotential and interferon induction.

Cancer chemotherapy, which perhaps unjustifiably dominated the meeting, covered conditions (such as lipid composition) under which liposomal drugs (for example, cytosine arabinoside (E. Mayhew, Roswell Park Memorial Institute; T. Kataoka, Japan Foundation of Cancer Research, Tokyo) or methotrexate, (H. K. Kimelberg, Albany Medical College)) given to ascites tumour-bearing mice prolonged survival more than did the free drugs. Although reduction of toxicity to normal tissues was a possible reason (Y. E. Rahman, Argonne National Laboratories) others, such as slow sustained formation of a more active metabolite (Ara-C triphosphate, Buffalo group) or sustained release of the drug itself were also thought likely. In connection with the possible treatment of lung metastases, markedly retarded removal of drugs from the lungs of animals given liposomal drugs by way of the airways was reported by R. L. Juliano (Hospital for Sick

Children, Toronto). Furthermore, localisation in animal tumours of a liposomal marker given intravenously was seen after scanning (B. E. Ryman, Charing Cross Hospital, London).

Ways to smuggle poliovirus into virus-resistant cells (R. Taber, Roswell Park Memorial Institute) and of enzymes into enzyme-deficient cells (G. Weissmann, New York University Medical Center) with the help of liposomes were of great interest and uptake of enzymes could be directed into specific cellular regions by appropriately designed liposomes. *In vivo* however, selectivity is much more difficult to achieve, especially in view of hepatic and splenic involvement. Some of the many properties of liposomes which influence their redirection to alternative sites as well as the role of the 'travelled' biological space (blood for example) were discussed (G. Gregoriadis, Clinical Research Centre, Harrow). Small liposomes could localise in target (tumour) areas more efficiently and liposomal lipid composition is essential in promoting the entrance of insulin given intragastrically into the periphery or in producing a better immune response to diphtheria toxoid. Homing of liposomes to specific cells, previously shown to occur *in vitro* by way of anti-target cell immunoglobulins incorporated into the liposomal surface, and successfully applied to the selective stimulation of lymphocytes (W. E. Magee, University of Texas, San Antonio) was, however, marred *in vivo* by the excessive participation of the liver.

In spite of their almost unlimited potential, liposomes have scarcely been investigated in man and the only therapeutic attempts have been enzyme replacement therapy of lysosomal storage diseases. An infant with glycolipidosis type II was given liposome-entrapped fungal amyloglucosidase but died before long-term observations could be made (Ryman). In a case of adult Gaucher's disease (glycosphingolipidosis) afflicting the reticuloendothelial system (liposomes home there spontaneously) the patient was repeatedly given liposomal human glucocerebrosidase: β -glucosidase over an 18-month period. There was marked relief of pressure and associated pain in the lower abdomen presumably as a result of a reduction in liver size. Each treatment was followed by pain in the liver which was proportional to the amount of enzyme used but which was absent with 'empty' liposomes (Gregoriadis). The ethics of the use of liposomes in such clinical trials before extensive animal experimentation were questioned. However, in view of the routine use of (Intralipid) phospholipid in intravenous feeding, selected phospholipids are likely to be less toxic

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than many of the cytotoxic drugs already in use, and it is rather the unknown way in which liposomes mediate drug action (or even toxicity) that one should consider. The implications are that under appropriate conditions, studies on marker-bearing liposome distribution in man could be a harmless and informative exercise, and that liposomal toxicity should always be examined in conjunction with the administered drug. Even so, in the absence of suitable animal models, the use of liposomal therapeutic agents in man might be acceptable. This is the case, for instance, with some human enzymes which in their free form have not been associated with toxicity and have not shown any beneficial effect.

The general feeling at the end of the conference was that although liposomes were already established as tools in the study of cell behaviour, their successful application in medicine was still a matter of speculation. On the other hand, as conventional drug treatment has in many instances proved unsuccessful, controlled drug delivery appears to be a hopeful alternative. In this respect, it is anticipated that liposomes will play a prominent part. □

A new human hepatitis virus

from Arie J. Zuckerman

THE identification of specific viral antigenic markers of hepatitis A (infectious or epidemic hepatitis) and hepatitis B (serum hepatitis) during the past few years has enabled sensitive laboratory tests to be developed. These, in turn, have led to a better understanding of the epidemiology, pathogenesis, immunology and nature of these infections; and, in the case of hepatitis B established the significance of this virus in severe chronic liver disease including at the very least a strong association with primary hepatocellular carcinoma in tropical and some subtropical regions. In addition, the specific diagnosis of hepatitis type A and hepatitis type B has revealed a previously unrecognised form of hepatitis which is clearly unrelated to either type. It is now the most common form of hepatitis occurring after blood transfusion in some

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Polymer diffusion disentangled

from P. D. Calvert

IN 1971 de Gennes predicted that the diffusion coefficient of a polymer chain in a gel would be proportional to the inverse square of the molecular weight (M) and with some reservations extended this prediction to self diffusion of molten polymers (*J. Chem. Phys.* **55**, 572; 1972). At the time the only available data, from NMR measurements by McCall *et al.* (*J. Chem. Phys.* **30**, 771; 1959), gave an exponent of $-5/3$. In this issue of *Nature* (page 143) Klein presents data for the self diffusion of polyethylene which confirms de Gennes prediction.

Self diffusion is not really an important property of polymers and most diffusion measurements are for gases or other small molecules; previous work of Klein and Briscoe and others in this field was described in a recent *News and Views* (**269**, 290; 1977). Viscosity is, on the other hand, an important property since most plastics are formed by squirting liquid polymer through small holes. Both self diffusion and viscosity involve molecules moving relative to one another, so one usually expects the diffusion coefficient to be inversely proportional to the viscosity.

Diffusion and viscosity in dilute polymer solutions has been well explained using Rouse models, where the polymer chains are viewed as strings of beads joined by springs and acted on by the flowing solvent. Molten short chain polymers can be treated similarly and one expects and gets a viscosity which increases proportionally to molecular weight, that is to chain length. At a critical molecular weight characteristic of each polymer this tidy state of affairs stops, and melt viscosity starts increasing proportionally to molecular weight to the power of 3.4. The reason for this is that the chains become entangled and difficult to separate. Exactly how this works and why the exponent is 3.4 has never been properly explained, though people have managed to obtain the right answer by rather

unconvincing routes.

De Gennes approached this problem by considering a chain molecule moving through a cross-linked network, so that the moving chain must wriggle through the network in a snake-like fashion. De Gennes christened this motion reptation. In essence the network chains can be thought of as forming an elastic tube within which the free molecule is constrained to move. This idea can be extended to molten polymers if we allow that the tube itself can move and reform, so that chain motion will be a combination of motion within the tube and motion of the tube. Reptation, motion within the tube, gives diffusion dependent on M^{-2} and viscosity on M^3 (de Gennes *Macromolecules* **9**, 594; 1976). Motion of the whole tube was tackled by Edwards and Grant (*J. Phys.* **A-6**, 1169, 1186; 1973) who showed that this gave a diffusion dependence of M^{-3} and viscosity of M^3 , and thought that this motion would dominate in molten polymers. What we conclude from Klein's results is that the tubes do not seem to move and reptation dominates, at least in diffusion. However, this still leaves us with the mysterious $M^{3.4}$ dependence observed for viscosity.

The idea of entanglements arose when workers on rubber elasticity found that cross-linked polymers acted as if they contained more junction points than are actually put in. It is a convenient concept, but carries with it the notion that a few interactions between chains act as tie points which are important for their properties, whilst the vast mass of interactions have little effect. It seems that reptation may lead to entanglements becoming redundant. Their replacement with a more realistic description of friction between random chains would be a real improvement.

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areas of the world (*Report of the WHO Expert Committee on Viral Hepatitis*. Techn. Rep. Series, No. 602, 1977). Although there are as yet no specific laboratory tests for identifying this new form of hepatitis, recent studies nevertheless stress the importance of this infection.

Results obtained from several recent surveys of post-transfusion hepatitis in

the United States provide strong epidemiological evidence of 'guilt by association' of an infection of the liver referred to in the USA as non-A: non-B hepatitis. Perhaps the most convincing report has just been published by Hoofnagle and colleagues (*Ann. intern. Med.* **87**, 14; 1977) after retesting serum samples that had been frozen from transmission studies con-

ducted in volunteer male prisoners in the USA in 1951-54 as part of a study on means of inactivating hepatitis virus in blood. The viral aetiology of hepatitis was in fact established by successful transmission experiments to volunteers first in Germany in 1942, in Palestine in 1943 and later by more extensive studies carried out in Britain and in the United States. Because a wide range of laboratory and other animals including monkeys, pigs, gerbils, jerboas, desert rodents and others had been inoculated with negative results permission for human inoculation tests was sought and obtained (*History of the Second World War. United Kingdom Medical Series. Medicine and Pathology*, 252 (Eds MacNalty, A. S. & Zachary Cope, V.) HMSO, 1952). In the series of volunteer studies carried out in the USA in the early 1950s, serum samples from six blood donors, implicated in the transmission of post-transfusion hepatitis after a single unit transfusion, were each inoculated into groups of 10-20 male prisoners. Sera from five of the implicated donors induced hepatitis in recipients. The serum samples collected during these studies have now been tested by Hoofnagle *et al.* for evidence of infection with hepatitis virus type A and B, cytomegalovirus and EB virus. Two of the donor sera contained markers of hepatitis B virus and transmitted hepatitis B infection to all susceptible recipients, two of

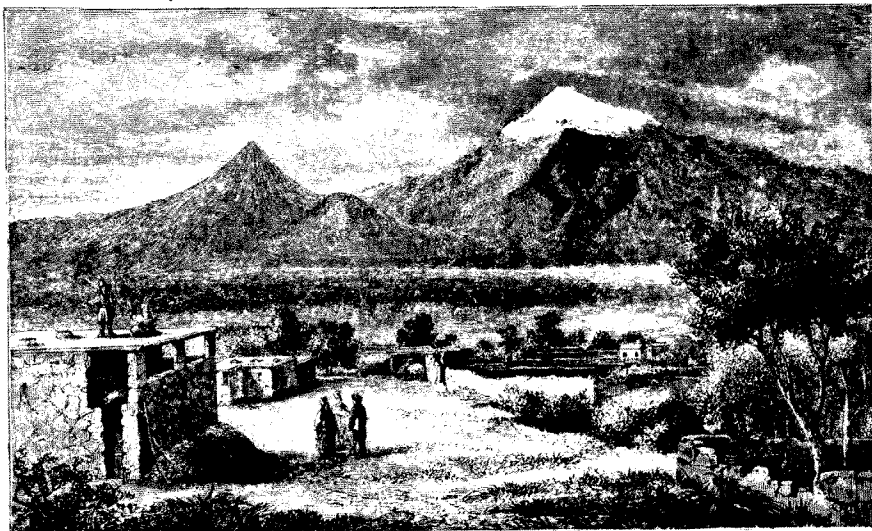
whom developed hepatic coma. The sera from the remaining three donors did not contain hepatitis B markers, but these sera were nevertheless infectious. A form of hepatitis was transmitted to nine volunteer recipients which could not be attributed to hepatitis A, hepatitis B nor to infection with cytomegalovirus or EB virus. The incubation period ranged from 18-89 d and the clinical illness was similar in most respects to hepatitis B. Of added significance was the finding of some evidence of persistent liver damage in one out of ten recipients who developed hepatitis type B and in two out of six volunteers who developed the unspecified type of hepatitis (non-A: non-B hepatitis).

In a recently completed survey on the efficacy of immunoglobulin (gamma globulin) for the prevention of post-transfusion hepatitis, Knodell and colleagues (*Gastroenterology* **72**, 902; 1977) identified 44 patients with acute non-A: non-B hepatitis. Sixteen of the patients had persistent biochemical evidence of liver damage 6 months after the acute illness. In 10 patients the abnormality of liver enzymes continued for 1-3 years and liver biopsy in each patient revealed histological evidence of chronic liver damage. It was further observed that 9 out of 22 patients who developed evidence of chronic hepatitis received albumin solution as a placebo, whereas only 1 of 11 patients who were given

normal immunoglobulin developed chronic hepatitis and none of 11 patients who received hepatitis B immunoglobulin. Further evidence for the existence of a third type of human viral hepatitis is provided by Dienstag and colleagues (*Ann. intern. Med.* **87**, 1; 1977) who investigated the cause of acute hepatitis in 45 patients admitted to a hospital in Los Angeles. There was no laboratory evidence of infection with hepatitis A, hepatitis B, cytomegalovirus or EB virus in 20 patients. It was noted that in nearly half of these patients infection followed the type of exposure traditionally associated with hepatitis B such as blood transfusion, plasmapheresis and occupational contact with patients.

These reports suggest the following: first, a carrier state of this newly recognised hepatitis agent since the infection is transmissible by blood transfusion (and probably by other routes) from apparently healthy donors; second, the infection must be common since pooled immunoglobulin seems to contain antibody as shown by the prophylactic efficacy of immunoglobulin in one study, and finally it seems that this infection may progress to chronic liver disease. The aetiological spectrum of viral hepatitis in man has thus been dramatically widened and there is an urgent need to develop specific laboratory tests for identifying this previously unrecognised virus or viruses. □

A hundred years ago



Great and Lesser Ararat from the North-east

So impressive a mountain, so long associated with man's faith and history, would have been appropriately placed among the most ancient landscapes of the earth's surface. Some scenes suggest only the changes of yesterday; others set us thinking of the earliest condition of our world. We naturally look for a kind of consonance between the venerable antiquity of the associations

which gather round Ararat and the primeval character of the finds, on closer research, that while most of these ridges have received their latest upheavals at a recent geological date, they yet for the greater part belong originally to earlier periods of disturbance, some of them, indeed, bearing witness to many successive uplifts during a vast section of geological time.

Yet further examination will bring before him evidence that along some of these lines of earth-folding, volcanic action has of old been abundant; and that the present Mediterranean volcanoes are but the lingering remnants of the chain of actively burning mountains which ran through Asia Minor and crowned the peaks of the Caucasus. And he will discover that just as there have been successive uplifts of the same axis or mountain-chain, so have there been widely-separated outbursts of volcanic activity during a long course of ages from the same focus of discharge.

In the middle distance is shown the alluvial plain of the Araxes. Below the snowy cone and icy cliffs of the Greater Ararat a deep cleft or recess appears with huge cliffs somewhat like the Val del Bove of Etna, and no doubt due to some of the volcanic explosions of the mountain. On the sky-line of this slope, towards the base of the larger cone, some of the late cinder-cones and craters appear. Some of these are still so fresh and perfect that they look as if they had been active only the other day and might blaze forth again tomorrow. The graceful outline of the Lesser Ararat rises on the left.

From *Nature* **17**, 10 January, 205; 1878.

review article

Chromatin

Gary Felsenfeld*

The approximate shape of the chromatin subunit called the nucleosome is now known, but its internal architecture is not well understood. Recent studies reveal details of the organisation of DNA within the nucleosome, and show that the arginine-rich histones are essential to DNA folding. Nucleosomes or structures related to them seem to be present at points of DNA replication and transcription; interactions within and between nucleosomes are likely to play a critical part in these processes.

RECENT studies of chromatin structure have focused on the nucleoprotein subunit called the nucleosome¹⁻⁹. Starting with this one certain structural element, investigators have worked in two directions, either examining the internal structure of the nucleosome, or speculating on ways of assembling arrays of nucleosomes to give the higher orders of structure and DNA compaction that are characteristic of chromatin in the nucleus.

The canonical nucleosome consists of a well defined length of DNA^{7,8} (about 200 base pairs) complexed with an octamer³ of histones. The octamer contains two copies each of the slightly lysine-rich histones H2A and H2B, and the arginine-rich histones H3 and H4; H1 is not part of the nucleosome, but is associated with it. As new data have accumulated, these general features of the nucleosome model have been confirmed, but the details have, not unexpectedly, been modified. For example, it is now clear that the amount of DNA per histone octamer can vary between about 140 and 240 base pairs, depending upon the organism and tissue from which the nucleosomes are isolated¹⁰⁻¹⁶; even within a single cell type, the spacing is not homogeneous^{15,17-19}. The evidence for this variability comes from studies in which nuclei are digested briefly with staphylococcal nuclease. The first points of attack are within the sensitive DNA 'spacer' or 'linker' regions separating the repeating units. This limited digestion releases nucleosome oligomers and monomers that are close to full-sized, and extrapolation to zero digestion time gives the size of the fundamental repeat. It is the repeat measured in this way that is found to vary with the source of the nuclei. Further digestion results in rapid preferential degradation of spacer DNA, leading, in the case of the monomer, to formation of a relatively stable nucleosome 'core' containing the histone octamer and a DNA segment 140 base pairs in length²⁰⁻²². The size of this segment is invariant in all chromatin samples so far examined^{10,12,15}. The variability of the length of DNA in the nucleosome repeat thus derives entirely from variation in the length of the spacer regions that separate the cores.

The earliest physical studies of nucleosome core structure suggested a particle roughly spherical in shape, about 100 Å in diameter. There was also a proposal, however, that the nucleosome was disk-shaped²³. Neutron scattering studies, which measured the contribution of the DNA to the radius of gyration of the particle, unambiguously placed the DNA at the exterior of the nucleosome^{6,24,25}. Recently, detailed study of the neutron and X-ray scattering behaviour of nucleosomes in solution has

led investigators at Searle²⁶⁻²⁸ to conclude that the nucleosome could not be spherical. Their data were fitted best by a flattened cylindrical structure about 100 Å in diameter and 50 Å in height, with the DNA wrapped around it to form a pair of rings at the top and bottom.

Though solution scattering studies can eliminate possible models of the core shape, they cannot be used to prove them unambiguously. Fortunately, more direct information about nucleosome core shape has now been provided by the work of Finch, Klug, and their collaborators²⁹, who have crystallised nucleosome core preparations and carried out combined X-ray diffraction and electron microscopic measurements with a resolution of 20 to 25 Å. Some caution is necessary in comparing their results with those from neutron scattering, since the crystallised core particles contained histones that had undergone partial proteolysis. However, there is reason to think that this results in only minor changes in structure. The crystallographic data show that the nucleosome core is a flat disk about 110 Å in diameter and 57 Å in height, in excellent agreement with the neutron scattering model. Given the present resolution, the exact path of the DNA cannot be determined, though the electron density map is not inconsistent with the location of DNA on the periphery of the particle, as demonstrated earlier by neutron scattering. If it is assumed that the 140 base pairs of DNA form a uniform superhelix wrapped around the cylinder, the superhelix must have a diameter of about 90 Å and a pitch of about 28 Å, corresponding to between 75 and 82 base pairs per superhelical turn of B-form DNA.

Action of nucleases on the nucleosome core

Additional inferences²⁹ about the structure can be made by combining information from diffraction with data obtained from nuclease digestion studies. It has been known for some time that the DNA of the nucleosome is susceptible to internal cleavage by nucleases. Although the spacer DNA is the preferred target, the nucleosome core is also attacked, at a slower rate^{30,31}. Staphylococcal nuclease cuts across both of the DNA strands³⁰, whereas other enzymes, such as pancreatic DNase, usually make single-strand cuts, so that the cutting pattern is somewhat obscured unless the digestion product is denatured³¹. Although the details of the process differ, all of the enzymes produce an array of fragments of discrete size. The pattern of single-strand cuts generated by pancreatic DNase is especially striking: when DNA from a partial digest of nuclei is denatured and subjected to gel electrophoresis, a series of discrete bands is observed, corresponding to DNA sizes that are integral multiples of ten nucleotides³¹ (Fig. 1, left). It is evident that there are discrete cleavage sites within the nucleosome; the way in which

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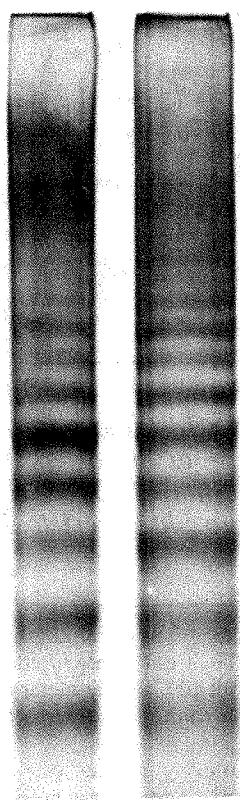


Fig. 1 The products of internal cleavage of nucleosomal DNA. Duck erythrocyte nuclei were digested with nuclease and the DNA purified, denatured, and electrophoresed on a polyacrylamide gel. Left channel: Pancreatic DNase (DNaseI) digest, 23% of the nuclear DNA acid soluble (after ref. 31). Right channel: Spleen acid DNase (DNaseII) digest, 60% acid soluble. Migration is from top to bottom. The dark-stained bands correspond to single strand DNA fractions separated by 10 nucleotide intervals in the approximate range 140–30 nucleotides. (From ref. 60.)

band intensities vary shows that the sites are not equally accessible.

The electrophoretic pattern is a map of the relative frequency of a given separation between sites, but it tells us nothing about their absolute location. For that, the nuclease digestion experiment is repeated starting with nucleosome core particles that have been labelled *in vitro* with ^{32}P at the 5' termini of the DNA chains. The electrophoretic pattern of radioactively labelled fragments reflects the distance of susceptible sites from a fixed point on each chain. This experiment has been carried out independently in three laboratories with roughly comparable results^{32–34}; there is general agreement that the sites 30, 80, and 110 nucleotides from each 5' terminus are quite insensitive to attack by pancreatic DNase. The absence of any fragments of these sizes means that each chain of the duplex is resistant to attack at these distances from its 5' terminus. This suggests a symmetrical distribution of protein–DNA contacts as a function of distance from the 5' termini of the antiparallel strands^{32,33}.

Such a symmetrical distribution of cleavage sites would be expected in a structure containing a dyad axis, as has been proposed on the basis of histone interaction studies³⁵. The possibility of this two-fold symmetry in the histone octamer itself has led to the proposal that the nucleosome not only contains a dyad axis, but is capable of unfolding into two symmetrical half-nucleosomes, perhaps as part of some mechanism of replication or transcription³⁶. A dissociation pathway of this kind is suggested by studies of histone interactions in the absence of DNA. When nucleosomes are dissolved in 2 M NaCl, the liberated core histones (H2A, H2B, H3, and H4) have been reported to form 'heterotypic tetramers' containing one molecule of each histone species³⁷. However, there is an unresolved disagreement about this observation,

since other workers report that the predominant species in 2 M NaCl is an octamer, rather than a tetramer³⁸.

Regardless of the nature of these histone interactions, the idea of nucleosome unfolding is supported by a number of studies of the effects of ionic strength and denaturing solvents on the nucleosome's physicochemical properties^{39–42}. Furthermore, it has recently been reported that at low ionic strength, the nucleosome as seen in the electron microscope appears to split into a pair of smaller particles⁴³. For example, the normal SV40 minichromosome, which bears 20–24 nucleosomes⁴⁴, is converted into a structure with a slightly greater contour length, but with 40 to 50 beads, each about 3/4 the diameter of the normal nucleosome.

Further evidence for a bipartite nucleosome structure comes from studies of the action of spleen acid DNase, a DNaseII, on chromatin. This enzyme can act on nuclear DNA to generate fragments that are multiples of 100 base pairs in length, suggesting that in addition to preferred cleavage sites between nucleosome cores, there may be another in the middle of each core. Although no corresponding nucleoprotein particle has been isolated, the results are consistent with the presence of a site of symmetry within the nucleosome. Preferential cleavage at this site is not seen in isolated nucleosomes, and only occurs in certain ionic conditions in nucleosome oligomers or purified chromatin; the inference can be made that nucleosome interaction and higher order structure are connected with this phenomenon.

Although the existence of a dyad axis in the nucleosome is not proven, it is certainly reasonable to introduce this symmetry element into any tentative model, and Finch *et al.*²⁹ have done so (Fig. 2). In addition, they have made use of the nuclease digestion studies mentioned earlier^{29,33}, which show that most sensitive sites, measured from a labelled 5' terminus, occur in pairs separated by 80 nucleotides along a given chain. Such a pattern could be explained if each superhelical turn contained exactly 80 base pairs, so that the backbone elements of adjacent turns were closest to one another when separated by 80 base pairs along the double helix. Since in this model the adjacent turns are separated by only 28 Å along the nucleosome surface, it is argued that the local environment, and hence nuclease sensitivity, would be the same for two elements separated by the length of a supercoil turn (Fig. 2).

This analysis is an attempt to explain the modulation of site accessibility, but avoids the more difficult question: what is the origin of the restriction that confines nuclease site separation to multiples of ten nucleotides? Two kinds of explanation have been proposed; both are related to the 10 base pair helical pitch of B-form DNA fibres, and both postulate that this periodicity exists in nucleosomal DNA. In the 'kinky helix' models^{16,47}, linear segments of B-form DNA are discontinuously bent at intervals of ten base pairs; the nuclease-sensitive sites are at the bends. A second model for DNA folding involves continuous bending of the DNA in such a way that the double

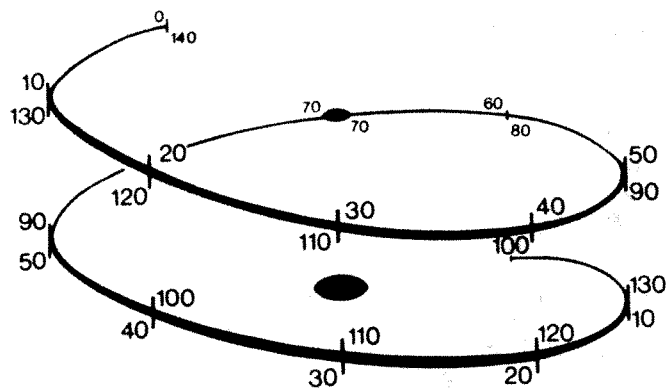


Fig. 2 Proposed superhelical path of DNA wound around the nucleosome core, showing nuclease cleavage sites and proposed dyad axis. (From ref. 29.)

helix makes one complete turn, relative to the nucleosome surface, for each ten base pairs. If a strand is accessible to nuclease only when it protrudes most from the nucleosome surface, nuclease-sensitive sites will occur once every ten nucleotides on each strand³¹. If cleavage occurred precisely at these sites, cuts on opposite strands would be separated by six or four nucleotides in a staggered arrangement. On the other hand, cuts occurring at kinks would be found either directly across from one another, or separated to give a stagger of ten nucleotides. These predictions can be tested directly by isolating double-stranded DNA from pancreatic DNase (DNaseI) digests. Wherever cuts on opposite strands are close together, fragments separate from each other, forming a series of small double-stranded molecules with single-stranded tails. The size of these tails, and thus the size of the stagger, can be determined by repairing the single-stranded region with a DNA polymerase, which extends all chains having recessed 3'-OH termini. It is found in this way that the cutting pattern corresponds to a stagger of 8 nucleotides (3'-OH recessed) and 2 nucleotides (5'-P recessed) between adjacent cutting sites on opposite strands^{18,19}.

This result is consistent with neither of the predictions described above. It can be made consistent with either by assuming that the site on the nucleosome recognized by the enzyme does not coincide with the cutting site, but is appropriately displaced in the 3' or 5' direction (Fig. 3). Further support for this idea comes from similar studies⁵⁰ of the mode of action of spleen acid DNase, a DNaseII. The nucleosome digest pattern of this enzyme is like that of DNaseI (Fig. 1), but detailed examination shows that in this case the cutting sites are staggered by six and four nucleotides (Fig. 3). Making use once again of labelling at the 5' terminus, the relative positions of the DNaseI and DNaseII cuts can be compared. It is found that the two sets of cutting sites are related to each other by a common dyad axis. Using somewhat different procedures, staphylococcal nuclease can be shown to cut at still another set of sites, which share the same dyad axis⁵⁰. It seems that the common recognition site is determined by the nucleosome structure, but the exact cutting point depends upon the enzyme.

Nucleosome folding and DNA supercoiling

Unfortunately none of this enables us to decide between kinked and continuously deformed models of DNA in the nucleosome. It is certainly possible to construct acceptable models of superhelical DNA by continuous deformation of the double helix^{29,51}. Estimates can be made of the energy required to bend DNA into a continuous supercoil with the dimensions of a nucleosome, using a well known relationship between the persistence length of such a stiff coil and its average bending force constant⁵². According to this model, about 20–28 kcal/mol of nucleosome are required to bend the DNA. The electrostatic forces which bind histones to DNA are very large at physiological ionic strength. The integrity of the folded nucleosome may therefore depend upon the attractive forces between histones within the nucleosome; if they are weak, the nucleosome will unfold. The free energy of interaction of histones in the nucleosome can be estimated, starting with free energy values measured in solution^{35,53}. It is found⁵² that one or two pairwise interactions of average strength would be sufficient to maintain the nucleosome in closed form. An unfolding pathway, such as that leading to half-nucleosomes, might involve disruption of just such a small number of interactions, and permit a delicate balance between folded and unfolded forms. It should be noted, however, that such an unfolding may not be an obligatory pathway for nucleosome assembly or disassembly: a particle with most of the physical properties of a nucleosome can be generated⁵⁴ by combining a DNA fragment 140 base pairs long with a histone octamer in which the proteins have been crosslinked, preventing histone dissociation and presumably interfering with unfolding as well.

Whether the DNA of the nucleosome is kinked or continuously deformed, every proposed structure must not only have

dimensions appropriate to the nucleosome, but must also satisfy the topological criteria implicit in experiments⁵⁵ which demonstrate the ability of the core histones to induce supercoiling. These experiments show that each octamer of core histones induces supercoiling in closed circular DNA equivalent to a change in linking number (ΔLk) of about -1 , reflecting additive contributions from changes in both the writhing number and the twist of the DNA^{56,57}. The simple models of kinking or continuous deformation described above fix the final state of twist of the DNA; similarly, the writhing number can be calculated for the proposed superhelical path of the double helix along the surface of the nucleosome²⁹. Thus, ΔLk can be calculated for the conversion of relaxed to nucleosomal DNA.

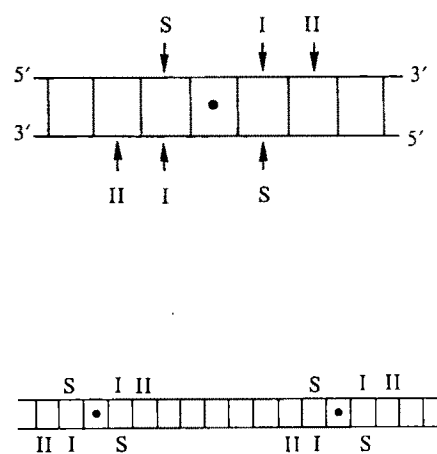


Fig. 3 Relative locations of the cleavage sites on nucleosomal DNA of pancreatic DNase (I), spleen acid DNase (II) and staphylococcal nuclease (S). The dot represents the local dyad axis of the cuts. (From ref. 50.)

If the fully relaxed conformation has ten base pairs per turn, as is generally assumed for B-form DNA, the agreement between the calculated and observed values of ΔLk is not good. It has been suggested²⁹, on the basis of recent theoretical calculations of DNA conformation, that B-form DNA in solution (though not in fibres) may in fact have about 10.7 base pairs per turn. This would certainly remove the discrepancy in ΔLk by changing the starting value of the twist, leaving the nucleosome model unchanged. At this point, none of the experimental observations concerning nucleosome structure, DNA supercoiling, or DNA conformation in solution is sufficiently precise or incontrovertible to permit a decision as to which is in error.

Role of arginine-rich histones

Nucleosomes can be reassembled by mixing DNA and histones under controlled conditions. The reconstituted particles have the characteristic beaded appearance in the electron microscope⁵⁵, and give typical subnucleosome fragment patterns when digested with nucleases^{30,58}. Recently, the four core histones have been reconstituted with DNA 140 base pairs in length, to give a homogeneous preparation of particles with sedimentation coefficient identical to that of native nucleosome cores⁵⁹.

The availability of reliable reconstitution methods makes it possible to examine the role of each histone component in the organisation of the nucleosome, by systematically omitting one or more from the reconstitution procedure. In this way it has been found that the arginine-rich histones, H3 and H4, are necessary and sufficient for the generation of a nucleosome-like complex. When H3 and H4 alone are reconstituted with DNA in appropriate conditions, the resulting complex can be digested with nucleases to give subnucleosomal DNA fragments identical in size (though not in relative abundance) to those produced when nucleosomes are digested^{58,60}. Such H3–H4 complexes also show a characteristic resistance of the histones to attack by trypsin or chymotrypsin, seen also in nucleosomes⁶⁰.

Furthermore, X-ray diffraction patterns of fibres made from reconstituted complexes of DNA with H3-H4 are almost identical to those obtained from intact chromatin fibres^{61,62}. It has also been shown that these two histones alone are capable of inducing an extent of supercoiling in closed circular DNA approximately equivalent in weight (roughly twice the normal complement) to that induced by all four histones^{62,63,64}. No combination of histones that omits either H3 or H4 gives any indication of ability to induce structure^{62,68,69}.

Complexes of the arginine-rich histone pair with closed circular DNA appear in the electron microscope as a beaded string^{63,64}, although the bead diameter is somewhat smaller than that of a normal nucleosome. If DNA 140 base pairs long is used, isolated beads are seen⁶³. The beads sediment in the ultracentrifuge in a manner consistent with their compact structure^{60,63}. Though there is some debate about whether a tetramer⁶³ or an octamer⁶⁰ of H3-H4 is associated with such a folded bead, it is clear that the arginine-rich histone pair plays a central part in nucleosome formation, as first suggested by Kornberg³. The arginine-rich histones have been the most strictly conserved in amino acid sequence during evolution. They are also the most tightly bound of all the histones. The results so far obtained leave open the question of the role of the slightly lysine-rich histones, H2A and H2B, in completing the normal nucleosome structure. That role may not become apparent until more is known about the function of nucleosomes in biological processes.

Higher orders of structure

The nucleosome must also be involved in the higher orders of structure that fold the DNA into the extremely compact form found in the nucleus. Attention has been concentrated on the first two levels of organisation, a 'thin' chromatin filament, 100 Å in diameter, and a thicker fibre, with a diameter of 200–300 Å. The thin fibre is almost certainly a linear array of nucleosome cores in contact with one another. The thick fibre seems to be generated by coiling of the thin fibre.

Neutron diffraction studies of the 300 Å chromatin fibre suggest that the nucleosomes are arranged in a solenoid with a 100 Å pitch, a diameter of 300 Å and a 100 Å hole down the central axis⁶⁵. The pitch is presumably determined by the side-by-side packing of nucleosomes on adjacent turns of the solenoid. Solenoids of these dimensions are also seen in electron micrographs of nucleosome oligomers⁶⁶, but only when magnesium ion is present. In its absence, only the 100 Å thin filament is visible. It is easy to build models^{66,67} in which strings of spherical beads are packed to form the solenoidal structures, although some modification will be necessary to take into account the correct, non-spherical nucleosome shape.

There is good reason to think that interactions between nucleosomes are modified or stabilised by the lysine-rich H1 histones. These histones are bound to chromatin at least in part through attachment to the DNA spacer region between nucleosome cores. During staphylococcal nuclease digestion, H1 histone is liberated from monomer nucleosomes as the DNA size is reduced from 200 base pairs to 140 base pairs^{22,68–70}. A DNA fragment about 35 base pairs long appears simultaneously, with H1 bound to it⁷¹.

The presence of H1 may have a direct effect upon chromatin structure. For example, SV40 minichromosomes containing H1 appear as compact structures in the electron microscope. Salt extraction, which should remove H1, allows the structure to open up into the more familiar beaded forms^{72–76}. Similarly, if well fractionated nucleosome oligomers are stripped of histone H1, there is a change in the dependence of the frictional coefficient on oligomer size⁷⁰, consistent with stabilisation of compact structures by H1. Studies of the dependence of H1 binding on the size of nucleosome oligomer show that there is a steady increase in affinity up to the octanucleosome, and no further size dependence beyond that⁷⁷. This suggests that octanucleosomes are capable of forming a stable unit of higher order structure: electron micrographs of oligonucleosome fractions

containing H1 reveal the presence of spherical structures about 200 Å in diameter, which have been termed 'superbeads', and seem to contain 6 to 10 nucleosomes each⁷⁷. The relationship of these structures to the superhelically wound oligonucleosome fibres seen earlier is uncertain. It has been pointed out⁷⁷ that exposure of chromatin to very low ionic strength solvents disrupts the higher order 300 Å chromatin fibre, and disturbs the size-selective binding of H1 noted above, perhaps by disruption of cooperative H1 interactions; variations in structure may arise from such rearrangements.

It is not clear exactly how H1 contributes to compaction of oligonucleosomes. The mode of binding of H1 to either negatively or positively wound superhelical DNA is different from its binding to the relaxed circular form⁷⁸. H1 may form bridges between superhelical turns, and indeed it has recently been shown that when H1 is added to H1-depleted SV40 minichromosomes, some H1 molecules link non-adjacent nucleosomes⁷⁵. H1 molecules may also connect spacer regions on either side of a single nucleosome⁷⁹. Either mode of binding could permit H1 molecules, if their termini were close to each other, to be polymerised by chemical cross-linking reagents, a phenomenon that has been observed⁸⁰.

Of all histones, H1 has the most variation in amino acid sequence among histone subspecies, as well as the largest number of variants. It is reasonable to speculate that the size of the variable spacer region between nucleosome cores is correlated with the subspecies of H1 bound to it; there may be a relationship between the size of the spacer and the number of basic residues on the H1 molecule^{10,14}. It has also been suggested^{81,82} that variation in the amino acid composition of histones H2A and H2B^{83–85} could alter contacts between nucleosomes, and affect the size of the spacer. Whatever the determinant of the spacer length, the thin filament and higher order structures must be capable of accommodating variable lengths of DNA in the region between nucleosomes. It can be inferred from nuclease digestion studies that in at least some cases the lengths of these regions are integral multiples of ten base pairs¹⁷, and may also have regularly spaced accessible sites within them when H1 is present. Worcel⁸¹ has proposed a model of the thin chromatin filament, 100 Å in diameter, in which adjacent nucleosome cores are in contact, but the cores can be rotated with respect to one another to permit inclusion of a variable length of spacer DNA without altering the diameter of the fibre. The spacer DNA is not in close contact with core proteins, but is assumed to be complexed with H1, in a way which stabilises supercoiling of the thin filament to form a 200–300 Å fibre. Furthermore, still higher order coiled structures can be formed from this fibre in such a way that the spacer DNA is always at the outside, and accessible to nucleases.

Such models, though they may turn out to be wrong in detail, provide important tools for thinking about higher orders of structure which nucleosome packing must generate, and which we have only begun to examine experimentally.

Chromatin structure and biological activity

The information we have so far obtained about chromatin structure clearly relates to DNA packaging in the eukaryotic nucleus. There is no reason to doubt the tenet of molecular biologists that knowledge of structure leads to understanding of function; what we now know about chromatin structure is simply not enough to permit the connection to be made. Perhaps for that reason, many of the questions now being asked about the role of histones in the biological activity of chromatin are little different in form from those asked four years ago⁸⁶, except that they are cast in terms of nucleosomes rather than in terms of more poorly defined histone complexes.

The obvious first task is to find out what happens to nucleosomes during DNA replication and transcription. In the case of replication, new histones are synthesised as well as new DNA, and new nucleosomes are made. Are the new histones segregated or mixed with the old? In a recent set of experiments⁸⁷, the fate of newly synthesised histones in chick myoblasts was traced by

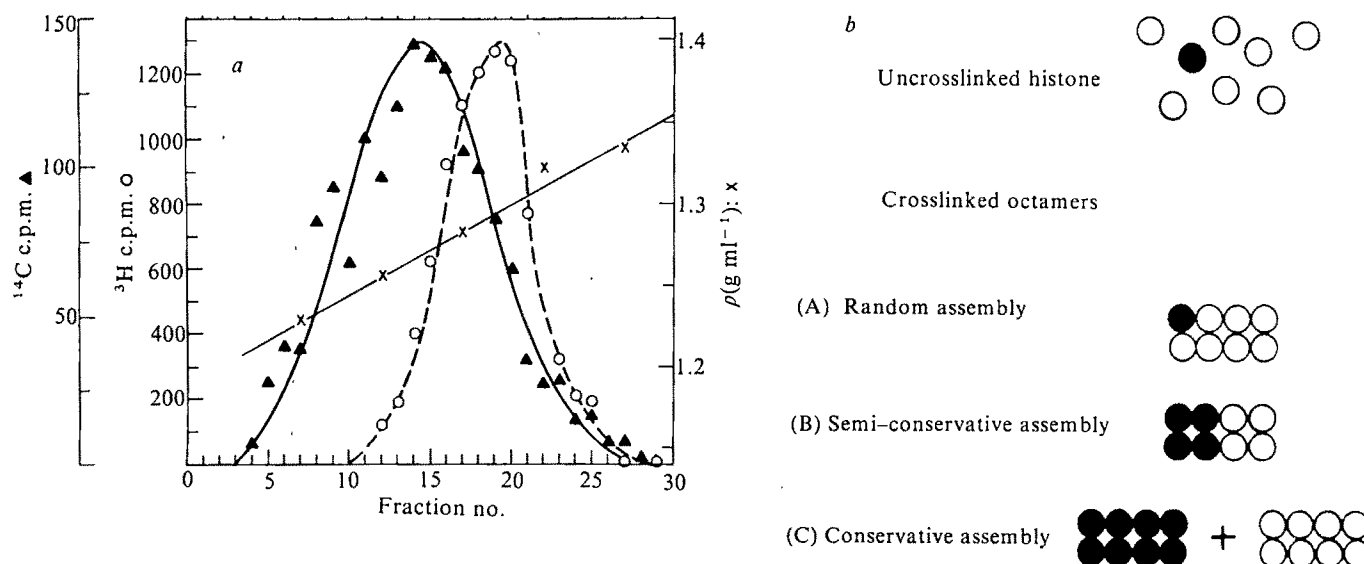


Fig. 4 *a*, Buoyant density centrifugation of newly synthesised histones crosslinked to form octamers. Cells were labelled for 1 h in dense amino acids and ^3H -lysine. Chromatin was prepared by mild nuclease treatment of nuclei. After crosslinking the histones with Lomant's reagent (dithiobis (succinimidyl propionate)), the sample was banded to equilibrium on a caesium formate-guanidinium chloride gradient, together with uncrosslinked, ^{14}C -lysine labelled, light histone marker. The density of the ^3H -labelled peak corresponds to octamers containing only dense amino acids. (From ref. 87.) *b*, Possible modes of assembly of newly synthesised histone octamers. The data in *a* support model C. (From ref. 87.)

pulse labelling them with ^3H -lysine and dense (^{13}C and ^{15}N labelled) amino acids. The nucleosome monomers were isolated from the cells, and the histones within each nucleosome cross-linked chemically. The crosslinked histones were freed from DNA and their density determined by isopycnic centrifugation. Although newly synthesised histone represented only one-seventh of the total histone, all the newly synthesised molecules were found to band in the fully dense position, showing that crosslinking within the octamer had not joined old and new histones to each other (Fig. 4). This demonstrates that newly synthesised histones form completely new nucleosomes, rather than forming mixed octamers with old histone.

By using modifications of this experimental approach, it is possible to show⁸⁷ that newly synthesised octamers tend to be located near other new octamers, confirming the model of non-random distribution of new nucleosomes that had been deduced earlier by less direct experimental methods^{88,89}. It can also be shown that individual histone octamers preserve their integrity (that is, segregate conservatively) through at least three or four rounds of replication⁸⁷. Other evidence suggests that such a conservative segregation mechanism governs the behaviour of long sequences of adjacent nucleosomes⁸⁷.

These experiments do not examine the way in which newly made histone interacts with newly made DNA, and in fact there are conflicting reports in the literature (see ref. 87) as to whether new histone distributes randomly, or segregates in association with new DNA. If the histone distribution is in fact non-random, the way is open to a variety of mechanisms which could, in principle, preserve the identity and sequence of nucleosomes in the chromatin of a stem cell line, while allowing diversity in daughter DNA duplexes.

Since histone octamers stay together *in vivo* through several generations, there is no need to invoke mechanisms in which the nucleosome disassembles as part of the replication process, though such mechanisms are not excluded provided that old subunits are able to find each other again. It remains to be determined how newly made nucleosomes choose the particular daughter helix to which they will bind. Whatever the role of nucleosomes in replication, it seems unlikely that their presence would result in a process simpler than that which occurs in prokaryotes⁹⁰. In studying replication in eukaryotes, it would be

reasonable to expect a complicated series of enzymatic reactions, to which nucleosomes have adapted.

Perhaps because the analogous reaction in prokaryotes is better understood, studies of chromatin transcription far outnumber studies of replication. It has proved difficult to construct a satisfactory transcription system *in vitro* that sheds light on the mechanism *in vivo*, partly because the mode of action of eukaryotic RNA polymerases is not well known. The prominent exception is the system for transcription of the 5S RNA genes of *Xenopus in vitro*. Roeder and his colleagues⁹¹ have shown that selective asymmetrical transcription of the 5S gene can be achieved, provided that the proper polymerase is used (that is the class III eukaryotic RNA polymerase, responsible for 5S gene transcription *in vivo*), and provided that the template is chromatin rather than protein-free DNA. Recent results⁹² suggest that reconstituted chromatin may be effective as a template, furnishing a tool for studying the role of the various components of chromatin in regulating transcription of these genes.

Most investigations of chromatin transcription *in vitro* have used bacterial RNA polymerase, and should be regarded as studies not of transcription, but of chromatin structure, using the polymerase as a probe. Although there have been numerous reports of selective gene-specific transcription of chromatin by bacterial polymerase, the approach has not so far yielded a great deal of information about chromatin structure, probably because the methods used are tedious and subject to numerous potential artefacts⁹³⁻⁹⁵. It has recently been found, for example, that when endogenous mRNA is present as a contaminant in chromatin preparations, it can be used as a template by *E. coli* RNA polymerase to make an RNA strand complementary to the message, which forms a duplex with it^{94,95}. Certain techniques for distinguishing and isolating newly made RNA, such as incorporation of mercury-substituted nucleoside triphosphates, will lead to isolation of the duplex, and hence accidental purification of the endogenous message^{94,95}. The endogenous message sequences will subsequently be detected in the assay and ascribed erroneously to synthesis *de novo*. Complications such as these have made it difficult to use bacterial RNA polymerases as probes of chromatin structure.

How can we obtain more information about chromatin

structure in the neighbourhood of a transcriptionally active gene? One useful approach is to ask whether the structure is like that of the bulk of chromatin with respect to its sensitivity to nuclease. The answer that is obtained depends upon the nuclease that is used.

When staphylococcal nuclease is used to digest transcriptionally active, non-ribosomal genes, the pattern of products is very similar to that generated by this enzyme when it attacks the bulk of chromatin. For example, globin genes in avian reticulocytes are protected against exhaustive digestion by staphylococcal nuclease to the same extent as all other chromatin DNA^{50,96,97}. This parallel behaviour is also observed earlier in digestion: Lacy and Axel⁹⁸ have isolated nucleosome monomers, sedimenting at 11S, from partial staphylococcal nuclease digests of avian reticulocyte nuclei, and find that the abundance of globin gene sequences in the DNA of this fraction is identical to the genomic abundance. Thus the globin genes in reticulocytes, which are active in producing globin mRNA, are packaged in structures possessing the same repeat as the nucleosome. Similar results have been obtained⁹⁹ for the abundance of ovalbumin sequences in purified nucleoprotein monomers from hen oviduct nuclei.

The distribution of ovalbumin sequences has also been examined by fractionating the partly digested nuclear DNA rather than the nucleoprotein oligomers¹⁰⁰. Early in digestion, most of the ovalbumin genes are present in DNA fragments that are multiples of a subunit about 200 base pairs long. But, the monomer DNA fraction is preferentially enriched in ovalbumin sequences. The apparent inconsistency with the earlier observations⁹⁹ that 11S nucleoprotein monomers are not similarly enriched would be resolved if some ovalbumin sequences 200 base pairs long were not derived from 11S particles. Proof of this hypothesis will require a careful accounting for the fate of all ovalbumin genes, both as monomer DNA and nucleoprotein, in the course of the digestion.

susceptibility of chromatin from adenovirus-transformed hamster cells, the easily digestible adenovirus sequences seem to correspond to that portion of the virus from which mRNA is transcribed; the other viral sequences are resistant.

These results reveal a correlation between DNaseI sensitivity and a potential for or history of gene activity. Response to the nuclease does not necessarily depend upon how often the gene is being transcribed. For example, the globin gene retains its sensitivity in blood cells from 18-d-old chick embryos⁹⁷, in which globin message synthesis has largely ceased. Similarly, gene sequences that are infrequently represented in the mRNA population are nonetheless DNase sensitive⁹⁸. If it is assumed that the abundance of an mRNA sequence reflects the number of RNA polymerase molecules bound to the corresponding DNA, it can be concluded that it is not the transcription complex that confers special sensitivity to pancreatic DNase. We do not know whether the structural differences detected by this enzyme are a cause or an effect of transcription.

What kinds of structure could confer such sensitivity? Protein-free DNA might be digested preferentially. However, the same sequences that are digested by DNaseI are resistant to staphylococcal nuclease in a way which indicates that proteins are present. It should be emphasised that the absence of preferential digestion by staphylococcal nuclease is not necessarily diagnostic of the presence of protein. For example, at very low ionic strength, the kinetic constants describing the action of this enzyme on chromatin and on naked DNA⁸⁰ are such that, in substrate excess, mixtures of chromatin and DNA will be digested at approximately equal rates. Thus, experiments which merely test the initial rate of digestion of a sequence may not, in certain conditions, distinguish between chromatin and DNA. For these reasons, the crucial observations with staphylococcal nuclease are the appearance of most of the transcriptionally active sequences in nucleosome-like repeats, and the appearance of the genomic abundance of these sequences in

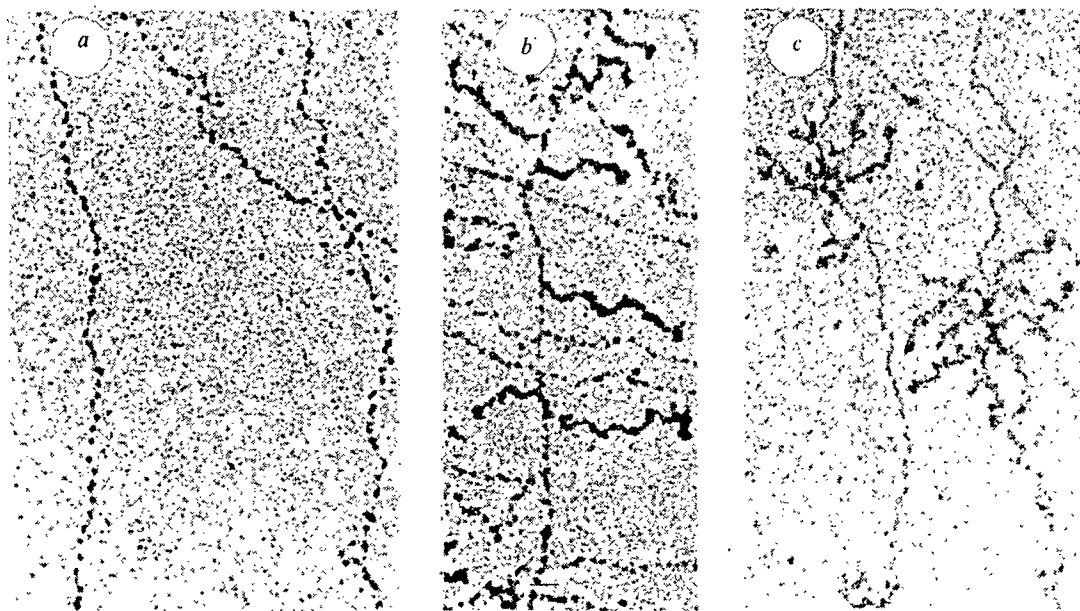


Fig. 5 Electron micrographs of chromatin from *Oncopeltus fasciatus*, enlarged about 67000 \times . *a*, Beaded chromatin from regions devoid of ribonucleoprotein (RNP) fibres. *b*, Beaded chromatin between nascent non-ribosomal RNP. *c*, Unbeaded chromatin of newly induced ribosomal transcription units. (From ref. 106.)

In contrast to the results with staphylococcal nuclease, the response of transcriptionally active sequences to digestion by pancreatic DNase (DNaseI) is strikingly different from that of normal chromatin. As first reported by Weintraub and Groudine⁹⁷, and subsequently confirmed in a number of laboratories, DNA sequences corresponding to active genes are digested preferentially. The list of susceptible sequences includes the globin gene in chick erythrocytes⁹⁷, the ovalbumin gene in hen oviduct¹⁰¹, and endogenous avian tumour virus sequences in chicken DNA⁹⁷. In a similar study¹⁰² of the pancreatic DNase

'limit digests'^{50,96}, which contain only fragments of subnucleosome size. The former observation shows that the DNaseI sensitive regions are packaged with proteins in a way that at least mimics nucleosomes; the latter observation strongly suggests that the proteins in question are indeed the nucleosomal complement of histones. To reconcile this conclusion with the observed sensitivity to pancreatic DNase, it must be assumed⁹⁷ that the nucleosome structure is modified or opened in a way that permits pancreatic DNase, but not staphylococcal nuclease, to detect the difference.

It should be noted that although the pattern of sensitivity described above has been observed for most of the active genes so far examined, there are exceptions. At least one integrated viral sequence has been found to be sensitive to both pancreatic DNase and staphylococcal nuclease¹⁰³. There is also some evidence both from nuclease digestion¹⁰⁴ studies and electron microscopy that actively transcribed ribosomal genes may not be like the other active genes discussed above. Ribosomal genes that are undergoing transcription are identified easily in the electron microscope by the characteristic tree-like structures formed by the growing ribonucleoprotein fibres, which extend from the DNA¹⁰⁵. Unlike the bulk of chromatin, no 'beads' are visible on these genes. Furthermore, since the length of DNA in each ribosomal gene is known, the DNA packing ratio can be measured, and it is found to be 1.2 (refs 106 and 107). Thus, the DNA of the transcribed ribosomal genes is only slightly less extended than normal protein free-DNA. This does not mean that proteins are necessarily absent from the ribosomal genes, since the mean diameter of the ribosomal chromatin fibre is about 73 Å.

Electron microscopists have also examined non-ribosomal genes undergoing transcription. Although subunit structure is not always visible¹⁰⁸, there are at least some cases in which a beaded structure has been reported¹⁰⁹. The difficult step in such observations is the identification of the active region. A tree-like structure of growing ribonucleoprotein chains can be found, and although it is less regular than the structure found on ribosomal genes, a convincing argument can be made that the chains arise from transcription of a delimited segment of DNA¹⁰⁶. Examination of these non-ribosomal 'transcription units' reveals the presence of beaded structures on the chromatin between ribonucleoprotein chains (Fig. 5). The diameter of these beads, about 13.5 nm, is similar to that found for nucleosomes under similar preparative conditions. The number of beads per unit length of chromatin within the transcription unit is about 70% that found in the bulk of chromatin¹⁰⁶. Recent experiments have shown that these regions react with antibodies to histones H2B and H3¹⁰⁹.

The electron microscope studies support many of the conclusions derived from the nuclease digestion experiments, but have not so far revealed the structural basis of sensitivity to pancreatic DNase. The sensitivity could arise from an alteration within each core particle (for example, to permit formation of half-nucleosomes), perhaps as a result of chemical modification of the histones, or it could reflect a perturbation of the higher-order packing of the nucleosomes, perhaps connected with loss or modification of histone H1 molecules.

The view we presently have of chromatin structure is still largely static. We do not yet understand any of the many conformational changes that must take place during the cell cycle both in individual nucleosomes, and in the higher order structures they form. Some of these changes may arise from modifications in composition. We know that the histones are chemically modified at various times during the cell cycle. Furthermore, during development, subspecies of certain histones are synthesised that differ slightly in amino acid sequence^{83,84} and might function differently in the nucleosome. Chromatin structure must also involve components that have been excluded from this discussion: the proteins that are not histones. The so-called high-mobility group (HMG) proteins¹¹⁰, for example, are found in abundance associated with chromatin¹¹¹. Another protein, ubiquitin, is found covalently coupled by an isopeptide linkage to histone H2A, in some cases modifying one in five H2A molecules^{112,113}. There are many other proteins, among them the polymerases themselves, that react with and may modify chromatin structure, and we may suppose that there are smaller molecules as well that have that capability. As we begin to understand the chemistry of these many reactions, the relationship between structure and mechanism may emerge.

I thank many colleagues for providing copies of manuscripts in advance of publication and Dr D. Camerini-Otero, Dr M.

Gellert, Dr J. McGhee and Dr M. Zasloff for their critical comments.

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articles

Volume and extent of the Minoan tephra from Santorini Volcano: new evidence from deep-sea sediment cores

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Analyses of tephra in abyssal piston cores collected during cruises of R/V Trident show that the Minoan eruption produced at least 28 km³ of tephra (13 km³ dense rock equivalent). A layer up to 5 cm thick must have been deposited on eastern Crete.

THE great Bronze Age eruption of Santorini (Thera) in the Hellenic arc is one of the largest known explosive eruptions in post-glacial time. The eruption, which occurred approximately 1500 BC, led to widespread tephra-fall in the Eastern Mediterranean and resulted in the formation of a caldera with an area covering 83 km² and a depth of 600–800 m. Apart from its volcanological significance, this Bronze Age eruption acquired added archaeological importance after Marinatos¹ advanced the theory that the wide-spread Late Minoan destruction on Crete was a consequence of the paroxysmal volcanic events on Santorini. The discovery of Minoan volcanic tephra in a few deep-sea sediment cores from the Eastern Mediterranean² demonstrated the large magnitude of the eruption and the extent of the tephra-fall, and excited closer examination of Marinatos' theory¹. In September 1975, 32 piston cores were collected from the Aegean Sea and parts of the Eastern Mediterranean (Fig. 1) during Cruise 172 of the RV Trident. These coring traverses were designed specifically to study the distribution and dispersal of tephra downwind from Santorini volcano. We report here results of our study on the Minoan ash layer which provide new evidence on the magnitude and effects of the eruption.

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Correlation and distribution of the Minoan tephra

The locations of the new cores, together with cores containing Minoan tephra from RV Trident Cruise 171 and Vema Cruise 10 are shown in Fig. 1a. The thicknesses of megascopically visible Minoan tephra layers are added together with isopach contours. Visible Minoan tephra was observed in 18 of the new cores. We have correlated the tephra layers not only from core to core, but also with the Minoan tephra sequence on Santorini itself by electron microprobe analyses of glass shards and pumice clasts. Table 1 shows mean analyses of the Minoan tephra as sampled on Santorini and mean analyses of glass shards from the Minoan layer in abyssal cores collected during the cruises of RV Trident and RV Vema. Figure 2a shows plots of FeO*–K₂O–CaO + MgO of microprobe analyses of glass in six of the largest pyroclastic deposits exposed in the caldera wall of Santorini. Figure 2b shows the same plots for five prominent megascopically visible tephra layers in the Trident and Vema core V10-58. We have also analysed many other smaller, less widespread layers on Santorini and in the cores and the Minoan tephra is always distinguishable on major element glass chemistry. The data in Table 1 and the figure demonstrate clearly that the major element glass chemistry of the Minoan tephra is sufficiently distinctive to facilitate easy identification of the ash. The data provide unambiguous intercore correlations and confirm earlier correlations^{2,3}. Doubts about the correlation of the Minoan tephra in deep-sea cores can now be discounted^{4,5}.

Our results on measured thicknesses of the Minoan tephra layer in the deep-sea cores are presented in an isopach map in Fig. 1a. The isopach contours are based principally on the observed thicknesses in the cores. We stress that the contours in Fig. 1a

depict minimum present-day thickness of compacted megascopic tephra layers in the cores. The new data indicate an easterly to southeasterly dispersal of the tephra with the fall-out axis passing through Karpathos. An even more easterly trend of the axis cannot be ruled out, however, on the basis of the present evidence. Isopach studies of the initial plinian deposit on Santorini itself⁶ have previously indicated a southeast to east-southeast dispersal during the early stages of the eruption. The direction of the

dispersal axis has been further confirmed by our continuing grain size studies of the tephra: the coarsest grain size in a tephra layer at a given distance from the source occurs close to our inferred axis (such as core 172-9) and the size decreases southwards. The extent of the tephra dispersal to the north is unknown due to lack of cores, but our data clearly show that some tephra-fall occurred on Crete and that the recent suggestion that the fall on Crete was negligible⁵ is untenable.

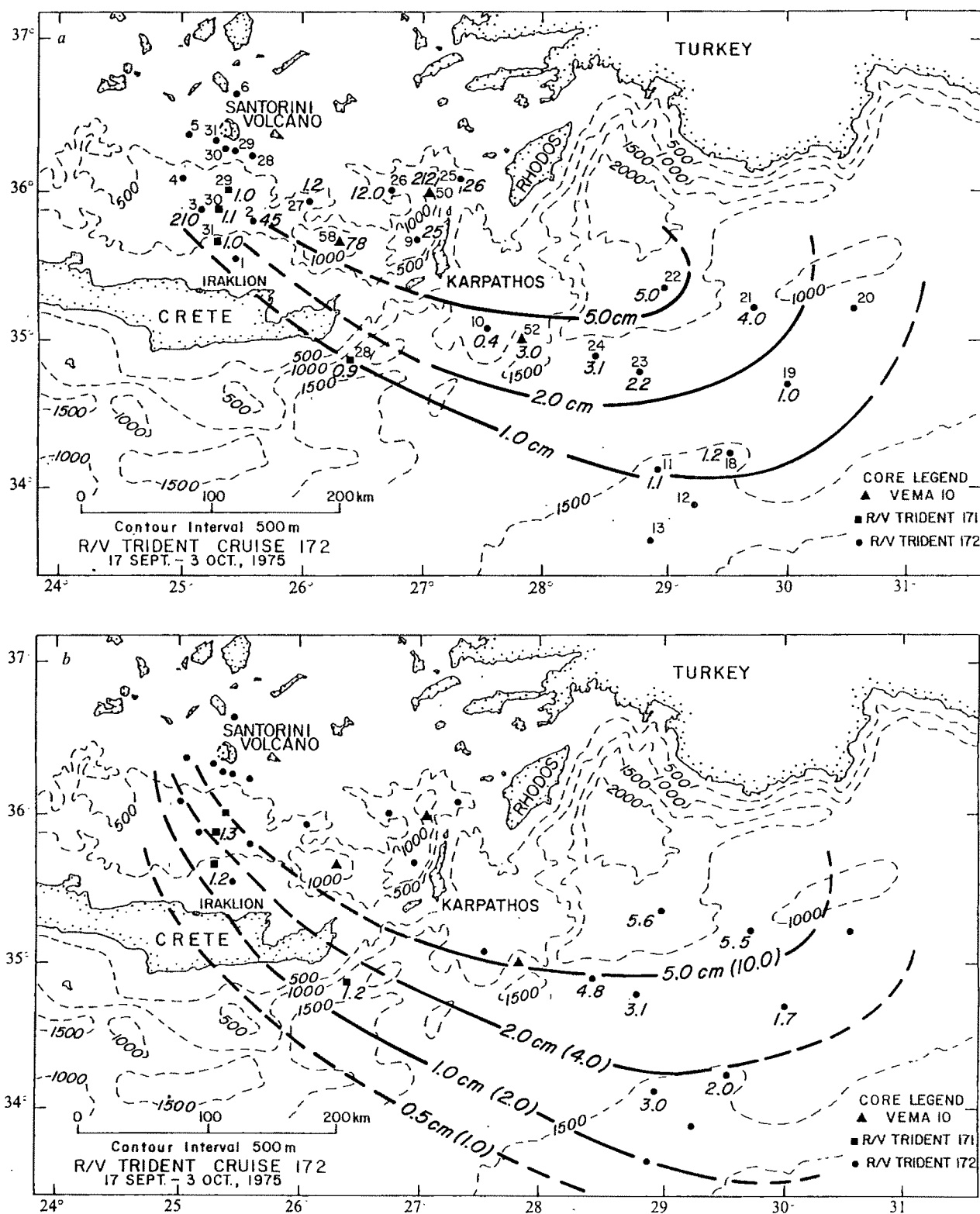


Fig. 1 Isopach maps of the Minoan tephra in Eastern Mediterranean cores. Isopachs are based on (a) the observed thickness (in cm) in the cores and (b) the adjusted thickness (in cm) to include the effects of tephra dispersal due to bioturbation. Note that in (a) the core numbers are given by small letters and the observed thicknesses in large letters. In (b) the numbers in parentheses on the isopach contours represent the thicknesses of freshly fallen tephra (before compaction) on land which are approximately twice those observed in the cores.

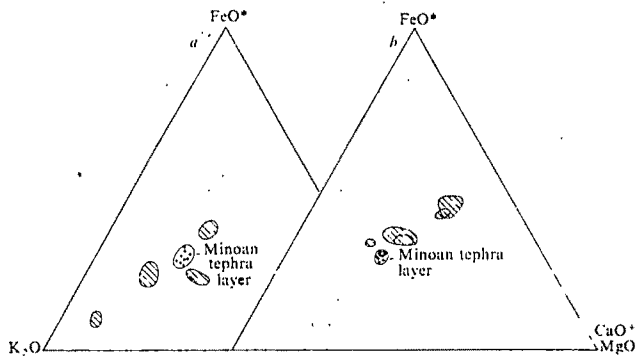


Fig. 2 FeO* (total iron) – K₂O – CaO + MgO plots of analyses of glass in tephra deposits from the Hellenic Island Arc. *a*, Six of the prominent pyroclastic layers in the caldera wall of Santorini are shown including the Minoan tephra layer. Each field is based on at least 10 analyses. *b*, Five widespread tephra layers found in cores from the Eastern Mediterranean are shown. The Minoan layer can be readily distinguished from the older Y-4 layer⁹ although the refractive indices are indistinguishable. Each field is defined by at least 12 analyses. The Minoan field in *a* coincides with that in *b*.

The RV Albatross cores collected by the Swedish Deep-sea Expedition⁷ are excluded from our data as we have been unable to obtain samples to correlate the alleged Minoan tephra layers in these cores. There is clearly some doubt over the correlations in the RV Albatross cores⁴: for example, core 192 south of Crete has a tephra layer in the upper part of the core with a thickness of 4 cm (refs 2,7) and has influenced previous reconstructions of tephra distribution and estimates of tephra thickness on Crete. This core lacks the distinctive 7,000 yr BP sapropel characteristic of many cores in the Eastern Mediterranean⁸. The stratigraphy in this core is therefore quite atypical of the strata associated with the authenticated Minoan tephra layer, but similar to the stratigraphy of tephra layers older than the 7,000 yr BP sapropel. An extensive tephra layer, known as Y-4 (after the recent stratigraphic nomenclature of Keller and others⁹), occurs beneath the sapropel in several cores and has a similar refractive index to the Minoan glass (1,508 ± 0.003). This layer, however is different chemically (Fig. 2*b*). The upper layer in the RV Albatross cores could equally well be the Y-4 layer if the upper parts of the cores are missing. Because of these uncertainties we exclude these cores until unambiguous correlations for tephra in the Albatross cores are produced.

Controls on tephra thickness

Tephra layer thicknesses have to be interpreted with caution as both volcanic and marine processes may control the ultimate thickness observed in a core. At least three important marine processes modify tephra thicknesses: sediment-ponding, bioturbation and compaction. When a large volume of loose tephra accumulates during an eruption, slumping may occur to generate turbidity currents, grain flows and debris flows. The consequence of such sediment-ponding processes¹⁰ is an increase in layer thicknesses in basins and to thin or erase layers on submarine topographic highs and steep slopes. Bioturbation of tephra layers mixes the tephra with other sediments and can reduce visible tephra layer thicknesses, dispersing tephra in the overlying sediment. Finally post-depositional compaction can substantially reduce the thickness of the tephra layer compared to the freshly-fallen thickness.

Within the Aegean Sea the great thicknesses within the basins are due to repeated slumping during the eruption to produce successive turbidite units. Evidence for turbidite origin includes erosional bases and partial Bouma sequences, including graded tephra beds. Figure 3 shows the lithology of several units within the Minoan tephra layer in core 172-9 as well as profiles of carbonate and clay content, median grain diameter, and pumice, lithic and crystal contents. The two layers labelled D are both interpreted as turbidites as they contain abundant carbonate, clay and some rounded terrigenous quartz grains, mixed in with the tephra. By contrast the tephra layer in core 172-27 (Fig. 1*a*), for example, is only 1.2 cm in thickness despite the fact that the core is

only 80 km away and lies close to our suggested dispersal axis. This core is located on a steep slope and we attribute thinning of the layer in TR 172-27 to the slumping or erosion of much of the tephra. For these reasons none of the measured thicknesses of the Minoan tephra within the Aegean Sea have been used in our construction of isopach contours.

Bioturbation affects most tephra layers, but is of particular importance outside the Aegean Sea where the tephra layer becomes relatively thin. Using our technique¹¹ we have quantitatively analysed the dispersed tephra in continuous samples taken above and below the visible tephra layer in 11 selected cores. The volume of dispersed tephra has been determined and its equivalent thickness added to the thicknesses of visible ash in each core. Up to 65% of original tephra has been mixed upward by bioturbation. In the Indian Ocean a 15 cm ash layer from Toba west of Sumatra has lost some 5 cm of ash by bioturbation into overlying sediment¹² emphasising the importance of this process. Fig. 1*b* shows the corrected tephra thicknesses and revised isopach contours. Comparison of Fig. 1*a* and 1*b* illustrates that tephra layer thicknesses can be markedly altered by bioturbation. While the reconstruction does not alter the basic dispersal pattern of the Minoan tephra, the corrections clearly make a substantial difference to volume estimates.

The thickness of a compacted tephra layer in a deep-sea core is considerably less than the thickness of freshly fallen tephra on land. We have studied the effect of post-depositional compaction on tephra layer thickness by experimental resedimentation of dry Minoan tephra from the cores. The results of three experiments in air show that the compacted tephra layers are equivalent to only approximately 50% of the thickness of resedimented dry tephra of equivalent weight and grain size. These results are in close agreement with our findings on the 1902 tephra of the Soufriere of St Vincent¹³. This eruption resulted in 10 mm of tephra fall on Barbados, whereas gravity cores recovered a 5-mm tephra layer in the adjacent Tobago Trough. Thorarinsson¹⁴ has reported similar relations from historic tephra layers in Iceland. In Fig. 1*b* we also show our estimates of the original thicknesses of freshly fallen tephra and reconstructed isopach contours. This procedure reveals the likely thicknesses of tephra on Crete and adjacent islands, although other factors causing tephra compaction under the deep-sea environments were not taken into account.

Eruption sequence and tephra volume estimates

Volcanological considerations prevent simplistic interpretations of the data. Recent studies of the Minoan deposits on Santorini^{6,15} indicate three main phases in the eruption: (1) the plinian phase producing a coarse tephra fall deposit; (2) a phase of phreatomagmatic activity producing interstratified fine grained and poorly-sorted tephra fall deposits and base surge horizons overlain by mud-flow deposits; and (3) a pyroclastic flow phase producing many non-welded ignimbrite flow units, intra-formatinal flood deposits and very fine grained co-ignimbrite

Table 1 Microprobe analyses of glass in Minoan tephra from Santorini and the deep-sea cores

Oxide	Tephra on land	σ	Tephra in cores	σ
SiO ₂	70.60	(0.18)	71.40	(0.10)
Al ₂ O ₃	13.60	(0.06)	13.50	(0.14)
FeO*	1.74	(0.06)	1.74	(0.05)
MgO	0.24	(0.02)	0.25	(0.02)
CaO	1.35	(0.01)	1.32	(0.05)
K ₂ O	3.12	(0.04)	3.19	(0.06)
Na ₂ O	5.07		4.98	
TiO ₂	0.26	(0.02)	0.27	(0.01)
Total	95.98		96.65	

The table shows the mean of six analyses for the land-based sample, and the mean of three analyses for the samples from cores, together with the standard deviation (σ) for each element oxide. All are expressed in weight %. Total iron is expressed as FeO*. All analyses were performed on a JEOL JXA-50A electron microprobe, with accelerating voltage of 15 kV, beam current of 0.0125 μ A, specimen current of 0.0100 μ A, and a beam diameter of 5–10 μ m. The rate of sodium loss during analysis was measured as 50% of original concentration in 30 s. The quoted values for sodium have been corrected accordingly.

tephra fall deposits¹⁶. Each of these phases produced a vertical convective eruption column and air-fall ejecta from each phase probably has its own dispersal pattern and grain size characteristics. Complexities in tephra dispersal have been observed in other eruptions such as Karmai (1912)¹⁷ and the Mount Mazama eruption, Crater Lake, Oregon¹⁸, which formed products comparable to the Minoan Eruption.

The tephra layer in core 172-9 (Fig. 3) compares with the stratigraphy observed on Santorini. A coarse, ungraded, well-sorted crystal-rich layer, 3.5 cm thick (B in Fig. 3) occurs at the base with a sharp upper boundary and is overlain by several fine grained layers (C-F). Two of the normally graded layers with erosional basal boundaries (Interval D) are turbidites. Evidence of mixing with local sediment is absent in layer B and it is interpreted as a primary air-fall layer buried by the turbidites and later fine-grained tephra fall. Although 165 km from source layer B lacks the fine grain sizes which are abundantly present in the tephra fall layers produced by phases (2) and (3) on Santorini itself⁶. Thus layer B is interpreted as correlating directly with the plinian layer (phase 1) on Santorini whereas the upper layers are predominantly composed of phase (2) and (3) ejecta probably mixed with minor amounts of phase (1) material during slumping.

Figure 4 shows the relation between thickness and distance in several well-documented eruptions together with the estimated volume of tephra in each case^{14,18-21}. If layer B (Fig. 3) represents the primary thickness of the plinian layer an approximate curve can be drawn for the Santorini plinian deposit, using the near source data⁶ and core TR 172-9. The plinian deposit falls between the Toluca deposit¹⁹ in Mexico and the Mount Mazama plinian deposit, Oregon¹⁸, suggesting an approximate volume of

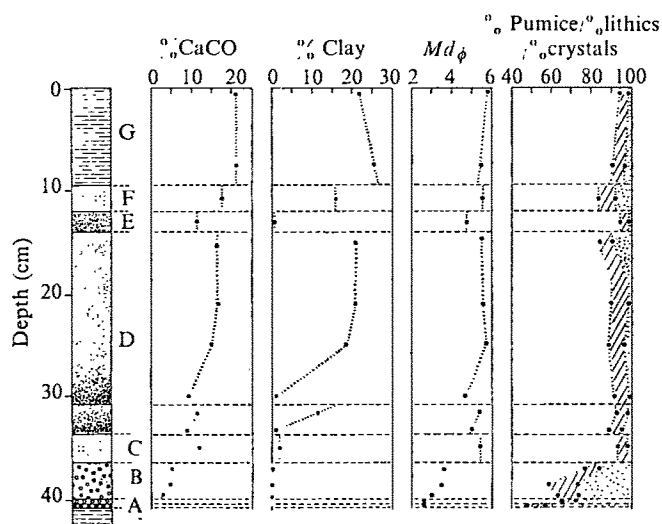


Fig. 3 Lithology of the Minoan tephra layer in core TR 172-9 showing sediment-ponding processes. From bottom to top: a, grain-flow deposit; b, plinian air-fall layer; c, fine tephra-fall unit; d, two repeated ash turbidites; e, and f, laminated tephra layers winnowed by bottom currents and g, pelagic muds admixed with tephra. Percent carbonate and clay, median grain size ($Md\phi$) and the weight percentage of pumice and glass shards (clear), lithics (hatched) and crystals (stippled) in grain size fractions greater than $63\ \mu\text{m}$.

5–8 km³ for the ejecta in this phase. To the east of Karpathos the tephra layer in all cores consists of a single layer and evidence of a multiphase eruption is absent. One possibility is that the plinian layer has thinned to such an extent that it is undetectable or even absent. The tephra layers at 350–500 km distance would then represent the equivalent of phases (ii) and (iii) of the eruption.

Whether or not the above interpretations are correct, the thicknesses east of Karpathos can be used extrapolated back to Santorini to give an approximate guide to the overall magnitude of the eruption, and to indicate the possible location of isopach contours in the Aegean (Fig. 4). The maximum thickness of all the air-fall layers on Santorini is about 15 m. This estimate is for the air-fall components of all three phases: individually the plinian pumice layer has a maximum thickness of 5.5 m, the fine-grained

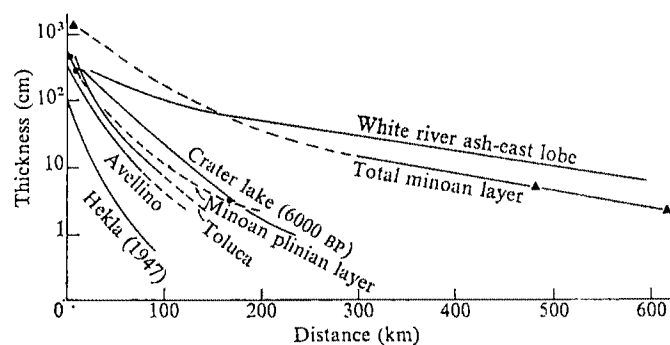


Fig. 4 Relationship between thickness and distance from source along the dispersal axis in several tephra fall layers, for comparison with the Minoan layer. Volumes of ejecta reported by the authors on each layer are Hekla¹⁴: 0.17 km³; Avellino pumice deposit, Vesuvius²⁰: 2.1 km³; Toluca deposit, Mexico¹⁹: 3.5 km³; Crater Lake airfall deposit, Oregon¹⁸: 15 km³; White River Ash²¹: 25 km³; Minoan tephra: 28 km³. Curves are shown for the Minoan plinian deposit (●), based on the interpretation of TR172-9 and for the compacted thickness of the total Minoan tephra layer (▲) using the adjusted isopach contours in Fig. 1b.

air-fall and base surge ashes a maximum thickness of 6.5 m and the co-ignimbrite air-fall ash deposits a maximum thickness of 3 m. We have used the data in Fig. 1b and the approximate distance/thickness curve in Fig. 4 to estimate the volume of tephra. We have assumed that the dispersal axis passes through Karpathos and that the volume of ejecta south of the axis is matched by an equivalent volume north of the axis. The volume of tephra on Santorini itself, assuming an average thickness of all tephra of 15 m over 200 km², is 3 km³ and we estimate the total minimum volume of the compacted deposit to be 28 km³, within the 0.5 isopach contour. We note that had we just used the uncorrected data in Fig. 1a the volume estimate would only have been 23 km³.

The bulk density of the abyssal tephra was determined as 1.0 g cm⁻³. The density of the original magma was calculated at 2.2 g cm⁻³ using the method of Bottinga and Weill²² for the average glass composition at a temperature of 850 °C, taking into account the 10% phenocrysts in the original melt and assuming a water content of 3.0%. Thus the dense rock equivalent volume (DRE) is estimated at 13 km³. There is a substantial discrepancy between the DRE volume and the volume required to account for the caldera (60 km³). We stress, however, that our estimate is very much a minimum for several reasons. Firstly, the volume outside the 0.5 cm isopach may be substantial; furthermore, the volume north of the dispersal axis is unconstrained simply because of insufficient data coverage and the axis could well be further north, leading to increased volume estimates. Finally a considerable proportion of the magma is believed to have been erupted as pyroclastic flows⁶ which formed substantial accumulations of tephra transported by turbidity currents into the basins surrounding Santorini. The number of available cores and their poor penetration close to Santorini is insufficient to estimate the volume of pyroclastic flow material.

Effects of the eruption on the Minoan civilisation

The new data provide improved constraints on the thickness of tephra which could have fallen on Crete. Figure 1b illustrates that the freshly fallen thickness probably fell in the range 0–5 cm. In eastern Crete, where the majority of Late Minoan settlements occurred²³, the thickness must have been between 1 and 5 cm depending on the locality. Very much greater thicknesses or very much less would be very difficult to reconcile with the data, and present understanding of tephra fall-out patterns. Such tephra thicknesses would undoubtedly have been an inconvenience to the Minoan population and could have produced some minor agricultural damage which may have been moderately severe in the extreme northeastern part of the island. Thorarinsson¹⁴ has documented that greater than 10 cm of freshly fallen tephra resulted in the abandonment of farms in Icelandic eruptions. We note, however, that agricultural techniques in Minoan times may have been less able to cope with tephra-fall than in more recent times.

Our data suggests that the Minoan colonies on Rhodes and the south coast of Turkey may have suffered severe tephra-fall, whereas tephra-fall on Crete was probably insufficient in itself to cause a major decline of the Minoan Civilisation. We emphasise that our data does not, however, preclude a relationship between the eruption and the demise of the Minoan Civilisation. Present understanding of the direct effects (for example, tephra-fall) and indirect effects (for example, tsunamis, earthquakes and meteorological phenomena) of very large explosive eruptions is very limited, as eruptions of this magnitude are yet to be chronicled.

This research is supported by NSF (grants OCE 74-22347, EAR 76-22083, OCE 75-21197 and OCE 72-01707). R. S. J. Sparks acknowledges a NATO post-doctoral fellowship. We thank the scientific parties and crew who helped collect the cores on RV Trident cruises 171 and 172. J. P. Kennett is thanked for the use of cores from cruise TR 171. Permission to study the Minoan tephra in Greek waters was obtained from the Greek authorities. Lamont-Doherty Geological Observatory Contribution No. 2556. Reviews by W. F. F. Ryan and F. McCoy are acknowledged. Norman

Watkins died on 2 November, 1977. The other authors deeply regret this tragedy to the man who inspired and led the present work on the Eastern Mediterranean.

Received 15 August, accepted 19 October 1978.

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Tumour-specific phenylalanine tRNA contains two supernumerary methylated bases

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Every malignant tumour examined contains aberrant tRNA methyltransferases and a few tRNAs which are absent from the normal tissue of origin. To determine whether tumour-specific tRNAs have different modifications from those in normal tissue, we purified the most frequently occurring tumour-specific isoaccepting tRNA from two malignant tissues. The isoaccepting phenylalanine tRNA from Novikoff hepatoma and Ehrlich ascites cells both contain two supernumerary methylated bases. One of these 1-methylguanine, is absent from the phenylalanine tRNA of normal rat, mouse, rabbit and calf liver. An increase in the levels of 5-methylcytidine and dihydrouridine was also detected.

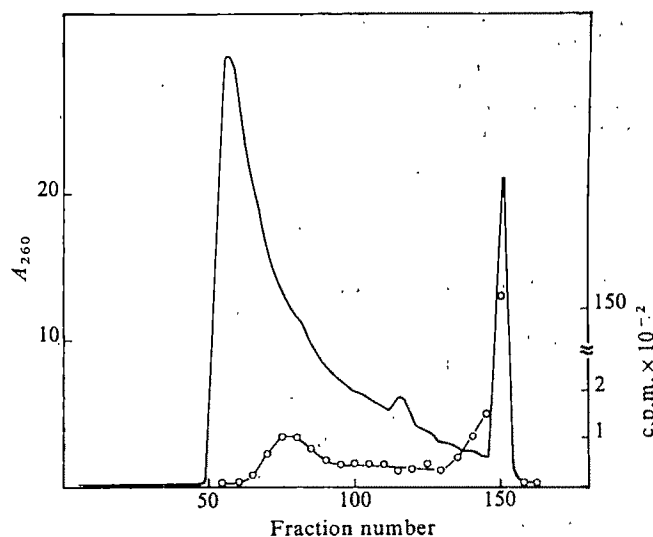
THE tRNA methyltransferases are aberrantly hyperactive in every malignant tumour examined and in each there are a few altered isoaccepting tRNAs which are absent from the tissue of origin¹. Whether the tumour-specific tRNAs differ in primary sequence or in modification has remained obscure. An analysis of the base sequence and of the modified bases in a pure, tumour-specific tRNA could establish the genesis of the altered tRNAs. If the primary sequence is found to be altered, it would imply that the tRNA originated either from a different or a mutated gene, or from error-prone transcription. If the primary sequence is unaltered, but some modifications are either absent or are supernumerary, then the tumour-specific tRNA is the product of aberrant modification.

There are conflicting reports in the literature about the modified base content of tRNAs in tumour tissue. Such confusion is not unexpected because until now, all the analyses have been performed on bulk tRNA and since most of the tRNAs in tumour tissue are identical to those of their normal counterparts, any changes in the few

tumour-specific tRNAs may be masked by the composition of the normal preponderant species.

Inspection of the literature reveals that the most frequently altered tRNA in tumour tissue is tRNA^{Phe}; therefore we undertook the purification of this tumour-specific tRNA from two different neoplasms, to compare

Fig. 1 BD-cellulose column chromatography of Novikoff hepatoma tRNA. The column (2×100 cm) was previously washed with 0.02 M sodium acetate buffer (pH 6.0) and 0.2 M NaCl. Novikoff hepatoma tRNA (10,000 A₂₆₀ units) was applied to the column. A linear gradient elution was carried out using 1 l of 0.02 M sodium acetate buffer (pH 6.0) and 0.5 M NaCl in the mixing chamber, and 1 l of 0.02 M sodium acetate buffer (pH 6.0) and 1.5 M NaCl in the reservoir. The column was washed finally with 0.02 M sodium acetate buffer (pH 6.0), 2 M NaCl and 20% v/v of ethyl alcohol. A volume of 10 ml of the effluent was collected per tube. —, Absorbance at 260 nm; ○, ³H-phenylalanine acceptor capacity.



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the base composition of it with that of pure tRNA^{Phe} from normal tissues.

Normal mammalian tRNA^{Phe} contains a highly modified hydrophobic base (O₂Y-Wye) (peroxywybutine) which is located adjacent to the 3' end of the anticodon^{2,3}. Consequently this tRNA has a unique behaviour on benzoylated DEAE-cellulose (BD-cellulose), since it is retained much longer than other tRNAs (ref. 3). Isolation of this tRNA is, therefore, relatively easy. The elution behaviour of tRNA^{Phe} from the tumours was found to differ from their normal counterpart, so a more elaborate purification procedure had to be devised.

Purification of tRNA^{Phe} from liver

Normal rat and mouse liver tRNA^{Phe} were eluted as a single peak from a BD-cellulose column at 2 M NaCl concentration containing 20% ethanol. Normal rat liver tRNA^{Phe} was further purified by fractionation on a RPC-5 column. A single peak of tRNA^{Phe} was observed and the

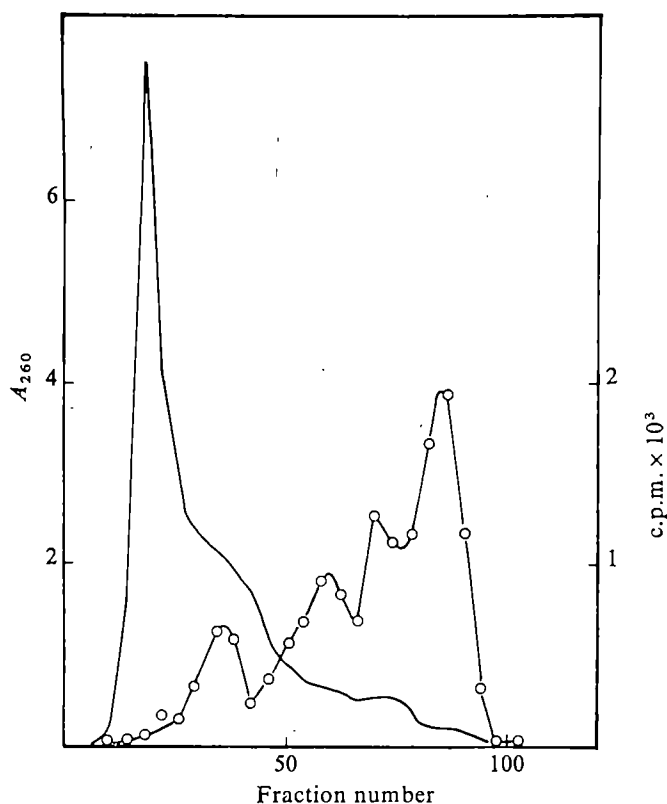


Fig. 2 Further purification of Novikoff hepatoma tRNA^{Phe} on a RPC-5 column. The column (1 × 50 cm) was previously washed with 0.01 M sodium acetate buffer (pH 4.7), 0.01 M MgCl₂, 2 mM β-mercaptoethanol and 0.4 M NaCl. The tRNA^{Phe}-rich region (750 *A*₂₆₀ units) obtained from a DEAE-Sephadex A-50 column (fractions 66–91, Fig. 1) was applied to the column. A linear gradient elution was carried out using 500 ml of 0.01 M sodium acetate buffer (pH 4.7), 0.01 M MgCl₂, 2 mM β-mercaptoethanol and 0.5 M NaCl in the mixing chamber, and 500 ml of 0.01 M sodium acetate buffer (pH 4.7), 0.01 M MgCl₂, 2 mM β-mercaptoethanol and 0.8 M NaCl in the reservoir. A volume of 5 ml of the effluent was collected per tube. —, Absorbance at 260 nm; ○, ³H-phenylalanine.

tRNA^{Phe} obtained from this column had an acceptance capacity of 1.36 nmol of phenylalanine per *A*₂₆₀ unit, indicating 82% purity. Purification of normal mouse liver tRNA^{Phe} was carried out using the same methods as on rat liver tRNA^{Phe}. Normal mouse liver tRNA^{Phe} purified this way accepted 1.51 nmol of phenylalanine per *A*₂₆₀ unit of tRNA, indicating 91% purity.

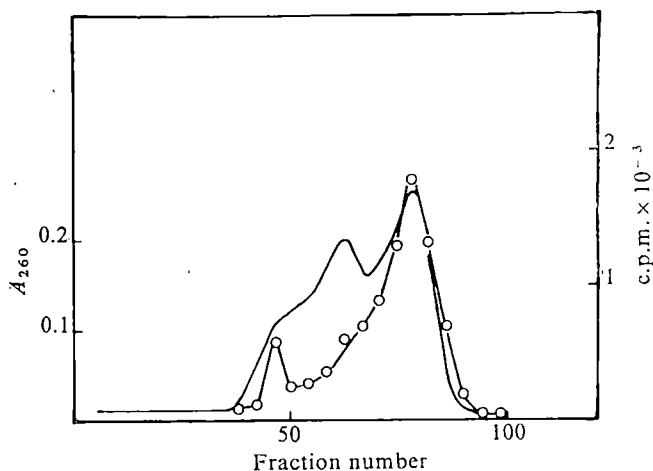


Fig. 3 Rechromatography of Novikoff hepatoma tRNA^{Phe} on a RPC-5 column. The conditions for chromatography were as in Fig. 2 except that 150 ml of the same acetate buffer in each chamber was used for elution. The tRNA^{Phe}-rich region (14 *A*₂₆₀ units) obtained from a RPC-5 column (fractions 76–95, Fig. 2) was applied to the column (0.6 × 30 cm). Each fraction contained 2 ml of the effluent. —, Absorbance at 260 nm; ○, ³H-phenylalanine acceptor capacity.

Purification of tumour-specific tRNA^{Phe} from two malignant tissues

Bulk tRNA was isolated from 1 kg of Novikoff hepatoma and was fractionated on a benzoylated DEAE-cellulose column by previously described methods^{4–6} (Fig. 1). The first minor peak, which was not detected on chromatography of normal rat liver tRNA, was further fractionated on a DEAE-Sephadex A-50 column. It eluted as a single peak and was further purified on a RPC-5 column. As seen in Fig. 2, the tRNA^{Phe} was resolved into at least three peaks. The last major tRNA^{Phe} (fractions 76–95) was rechromatographed on the same column (Fig. 3). Fractions 75–86 were pooled and precipitated by adding 0.1 vol 1 M

Fig. 4 Elution profile of phenylalanyl-tRNA of Novikoff hepatoma and normal rat liver on a RPC-5 column. ³H- or ¹⁴C-labelled phenylalanyl-tRNA was prepared by previously described methods⁷. Novikoff hepatoma aminoacyl-tRNA synthetase was used to charge the tRNA. The conditions for chromatography were as described previously⁷ except that a linear gradient of 0.5–0.85 M NaCl was used. *a*, ³H-Phenylalanyl-tRNA from unfractionated Novikoff hepatoma tRNA (○) and ¹⁴C-phenylalanyl-tRNA from unfractionated normal rat liver tRNA (●). *b*, ³H-phenylalanyl-tRNA purified from Novikoff hepatoma (—) and ¹⁴C-phenylalanyl-tRNA^{Phe} from unfractionated Novikoff hepatoma tRNA (○).

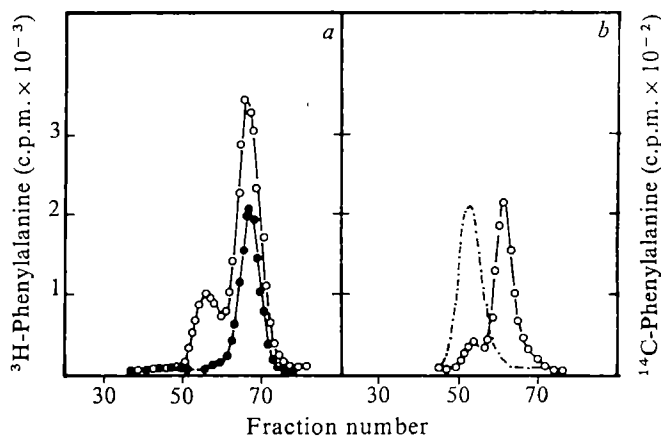


Table 1 Molar ratio of nucleosides of tRNA ^{Phe}						
Base	Mouse liver	Ehrlich ascites cells	Rat liver	Novikoff hepatoma	Rabbit* liver	Calf* liver
hu	2.7	4.0	3.1	4.2	2.2	3
m ¹ G	—	0.8	—	1.0	—	—
m ⁵ C	1.1	2.0	0.9	2.4	1	1
m ¹ A	2.1	2.1	2.0	2.0	2	2
T	0.6	0.5	0.8	0.6	0.7	0.5
m ₂ ² G	1.0	1.0	1.0	1.0	1	1
m ² G	1.1	1.1	1.1	1.2	1	1
Ψ	4.0	4.3	3.3	4.4	4	4
m ⁷ G	1.0	0.9	1.1	1.1	1	1
A	16.0	15.5	16.0	14.9	16	16
C	17.3	19.1	16.8	21.4	15	15
U	10.1	9.3	8.2	11.1	10	9.5
G	19.0	18.6	19.2	22.3	19	18

The tritium-labelled chromatographically separated trialcohols (as in Figs 5 and 6) were scraped from the plates, counted and their relative abundance calculated, assuming 1 mol of m₂²G per tRNA. That such an assumption is valid is indicated by the number of mol of nucleosides found: they range from 74 in tRNA^{Phe} from rat liver to 87 in Novikoff hepatoma. If two m₂²G were present, the number of mol of nucleosides would be twice those values.

* These data from ref. 9.

sodium acetate and 2.5 vol ethanol. Purified Novikoff hepatoma tRNA^{Phe} accepted 1.33 nmol phenylalanine per A₂₆₀ unit, indicating 80% purity.

Figure 4a shows the cochromatography on a reverse phase column (RPC-5) of phe-tRNA^{Phe} from unfractionated normal rat liver tRNA and from unfractionated Novikoff hepatoma tRNA. The cochromatography of the purified

phe-tRNA^{Phe} from Novikoff hepatoma (early eluting) with the unfractionated material is shown in Fig. 4b.

Bulk tRNA was isolated from 100 g of Ehrlich ascites cells, and a tumour-specific tRNA^{Phe} which had chromatographic behaviour identical to that of tumour-specific tRNA^{Phe} from Novikoff hepatoma was purified by the procedure described earlier. This preparation accepted

Fig. 5 Fluorograph of tRNA^{Phe} from *a*, normal rat liver and *b*, Novikoff hepatoma (0.2 A₂₆₀ unit). Purified tRNA^{Phe} was digested for 6 h at 37 °C with a reaction mixture containing 3 µg RNase A, 3 µg of snake venom phosphodiesterase, 2.4 µg of *E. coli* alkaline phosphatase, 0.01 M MgCl₂, 0.03 M bicine buffer (pH 8.0) in a final volume of 20 µl. After appropriate dilution, the enzymatic digests were subjected to periodate oxidation and reduction with ³H-KBH₄ to form the tritium-labelled nucleoside trialcohols. The tritium-labelled nucleoside derivatives were separated on cellulose thin layers in solvent A (Acetonitrile-4 N ammonia, 3.4:1, v/v) and solvent B (*n*-amyl alcohol-methylethylketone-acetonitrile-ethylacetate-water-formic acid, 4.2:1.5:2:1.5:0.18, v/v). The tritium-labelled compounds were detected on X-ray film by fluorography. The base composition was calculated as described by Randerath⁸. Abbreviations used: O₂Y-Wye, peroxywybutine; U', A', C', G', nucleoside trialcohols of uridine, adenosine, cytidine, and guanosine; m⁵U', m¹A', m⁵C', m²G', m₂²G', m⁷G', m¹G', nucleoside trialcohols of 5-methyluridine, 1-methyladenosine, 5-methylcytidine, 2-methylguanosine, 2,2-dimethylguanosine, 7-methylguanosine, and 1-methylguanosine; hU', Ψ', nucleoside trialcohols of dihydrouridine and pseudouridine; gly, glycerol; Ψ-D, Ψ-M, decomposition products derived from pseudouridine.



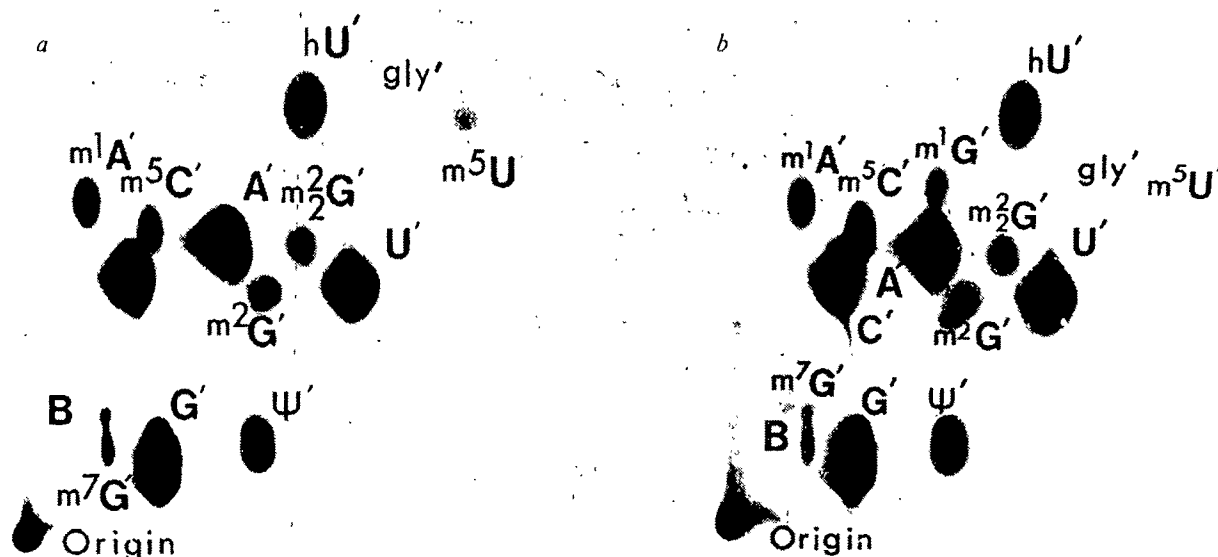


Fig. 6 Fluorograph of tRNA^{Phe} from *a*, normal mouse liver and *b*, Ehrlich ascites cells. The method and abbreviations are as used for Fig. 5.

1.48 nmol of phenylalanine per A_{260} unit of tRNA, indicating 89% purity.

Nucleoside composition of tRNA^{Phe} from tumour and normal tissues

Figures 5 and 6 show the nucleoside composition, determined by the method of Randerath⁸, of tRNA^{Phe} from normal rat liver (Fig. 5a), Novikoff hepatoma (Fig. 5b), normal mouse liver (Fig. 6a) and from Ehrlich ascites cells (Fig. 6b). The unique presence of 1-methyl guanosine in tRNA^{Phe} from both malignant tissues is noteworthy. This modification is also absent from tRNA^{Phe} from two other mammalian sources (Table 1).

Table 1 gives the molar ratios of the major and modified nucleosides to tRNA^{Phe} from four different normal mammalian sources and from the two malignant tissues. Two modified nucleosides, dihydrouridine and 5-methyl cytosine were found to be elevated by 1 mol in the tRNA^{Phe} from the malignant tissues; otherwise, the composition coincides to within experimental error with that of tRNA^{Phe} from other mammalian sources. The total number of nucleosides in tRNA^{Phe} from Novikoff hepatoma seems to be higher than in those from other sources. Whether this augmented structure is real will be resolved by sequence analysis which is under way.

Discussion

The findings reported here provide unequivocal evidence for aberrant modification of a tumour-specific tRNA, but this does not rule out the possibility of altered nucleoside sequence as well. The paucity of the supernumerary methylated bases is at first glance surprising, in view of the very high activity of the tRNA methyltransferases in malignant tumour tissues. Studies in our laboratory, however, have demonstrated a very high turnover rate of tRNAs of tumour tissue¹⁰. Whether excessively methylated tRNAs are rapidly eliminated remains to be determined.

Given the numerous and varied roles of minor species of tRNAs in other systems, it is difficult to predict what, if any, aberrant attributes these minor changes in modification may confer on the tumour cell. Ames *et al.* have shown that the lack of modification of two Us to ψ's in tRNA^{His} makes a profound impact on the economy of *Salmonella*. In the absence of the modification of tRNA^{His},

a mutant *Salmonella* is continuously derepressed for the production of nine enzymes in the histidine-synthesising pathway, even in the presence of an adequate supply of histidine¹¹. The consequent waste in energy and material for the cell is enormous.

A more recent implication of a role of methylation of tRNA for transcriptional control has been adduced by Freundlich and his coworkers¹², who found that if a methionine auxotroph of *S. typhimurium* was deprived of its essential amino acid, there were changes in the chromatographic profiles of tRNA^{Val}, tRNA^{Isoleu} and tRNA^{Leu}. Incomplete modifications by methylation were evidenced by the ability of the total population of tRNAs to serve as substrate for methylation by homologous enzymes. Concomitant to the changes in the tRNA elution profiles there was a 5–10-fold increase in the synthetic enzymes for isoleucine and valine even though an excess of these amino acids was available to the organisms.

The evidence for the loss of functional effectiveness in derepression by undermodified tRNAs is, at present, circumstantial. Perfection of appropriate systems of tRNA-dependent transcription is needed for unequivocal evidence for loss of regulatory capacity by undermodified tRNAs.

What functional capacity is endowed—or deprived—by aberrant modification of tRNA in tumour tissue is not known. As the reality of aberrant modification has now been established, however, a search for altered functions of such purified tRNAs can be undertaken with confidence.

We thank Dr B. A. Roe of Kent State University, Ohio, for instruction in the method of base analysis. The work was supported by a grant CA 14047 from the US NIH.

Received 13 August; accepted 1 November 1977.

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α -Helix-double helix interaction shown in the structure of a protamine-transfer RNA complex and a nucleoprotamine model

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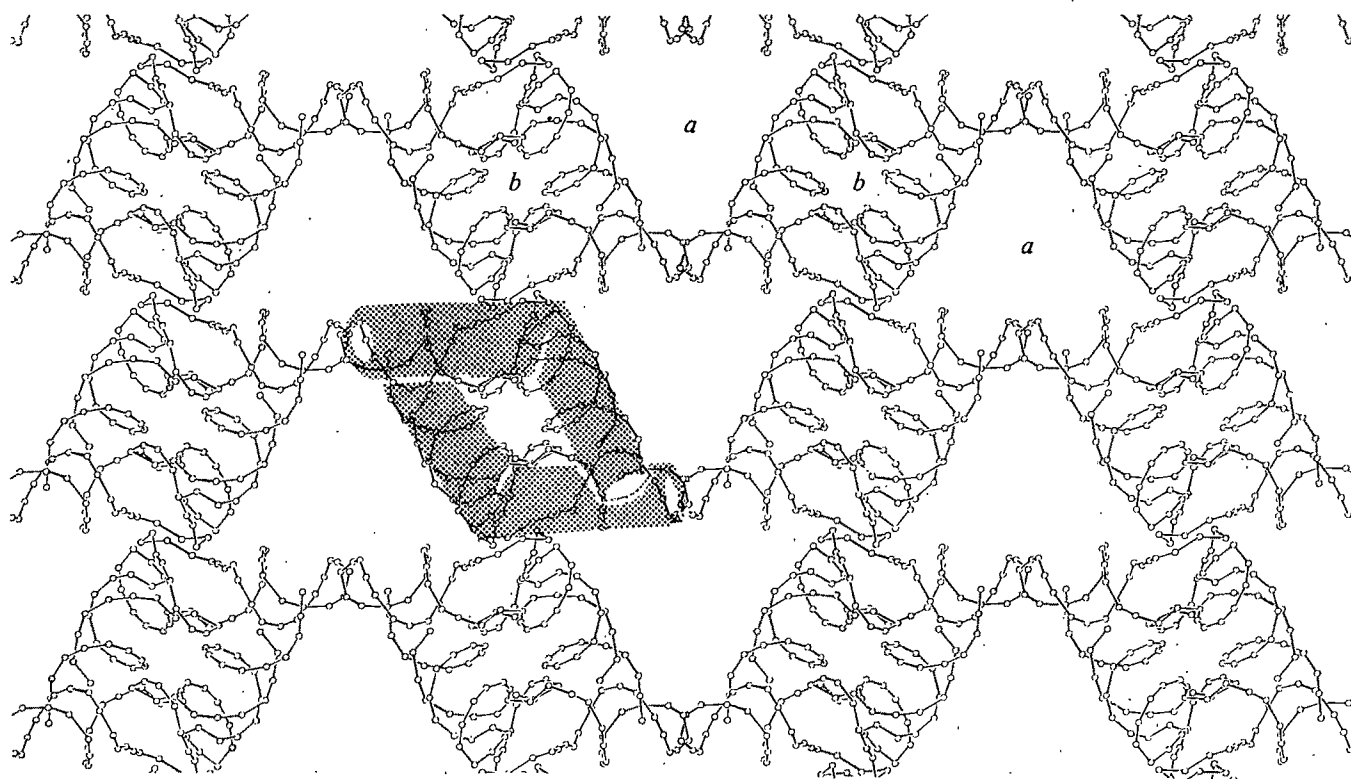
Single crystal X-ray diffraction and circular dichroism studies of protamine binding to a tRNA suggest that the protamine molecule changes its conformation from a random coil to a structure containing a helices on binding to tRNA, and that α -helical segment(s) of protamine bind approximately along a shallow groove of a double-helical portion of tRNA. Based on these observations, a structural model for nucleoprotamine is proposed.

PROTAMINES are extremely basic, small proteins of molecular weights of about 4,200. They are found tightly associated with DNAs in fish spermatozoa. Nearly two-thirds of the amino acid residues in protamines are basic, and these basic residues are usually found clustered, four or five in a sequence. When protamine messenger RNA (mRNA) reaches the cytoplasm, the residues are translated by diribosomes. The newly synthesised protamines are phosphorylated by ATP in cytoplasm,

catalysed by protamine kinase on serine residues. The phosphorylated protamines are transferred into the cell nucleus and then into chromatin, where they replace the histones and bind to DNA. During the maturation of the sperm heads, the protamine is dephosphorylated, the DNA becomes more condensed, and all transcription stops.

Much research has been directed to find the mode of binding between protamine and double-helical DNA by X-ray diffraction methods¹⁻³, spectroscopic techniques^{4,5}, electron microscopy⁶ and chemical modification⁷. The most widely accepted view at present is that protamine exists in an extended chain which wraps around double-helical DNA, covering the narrow groove along the DNA double helix^{1,2}, or sometimes crossing over to a neighbouring DNA double helix^{6,8}, with the positively charged basic side chains of protamine neutralising negatively charged phosphates of the polynucleotide chain. In a less specific model⁹, hexagonally arranged parallel rods of double-helical DNA are held together loosely by a network of protamines.

Fig. 1 Packing diagram of yeast phenylalanine tRNA in an orthorhombic crystalline form (P_{2121}) viewed along the crystallographic axis a . Two L-shaped tRNA molecules at an angle are shown shaded, and two types of channels are indicated. The large channels (a) have a diameter of > 40 Å, and the small channels (b) have a diameter of approximately 15 Å. This figure represents the thickness of the unit cell along the a axis. In the crystal, this packing is repeated indefinitely in all three directions, all exactly registered. In this crystalline form, the volume occupied by tRNA is approximately 25%, and that for the buffer is approximately 75%.



To understand the interactions between protamines and nucleic acids in more detail, we investigated crystallographically the interactions between protamines and transfer RNA (tRNA) primarily because tRNA has been crystallised in several suitable single crystalline forms, whereas DNA has not.

Yeast phenylalanine tRNA crystallises into an orthorhombic lattice, in which 75% of the total volume is occupied by buffer solution. There are two kinds of channels in this crystal form (Fig. 1). The large channel, *a*, is over 40 Å in diameter and the small channel, *b*, is about 15 Å in diameter. Thus, this crystal form provides an open crystalline matrix into which various molecules can be soaked, to study their interaction with tRNA by single crystal X-ray diffraction techniques. Since a large portion of tRNA structure is double helical, this system can be considered for studies involving double-helical RNA in general. We report here the crystal structure of a protamine-tRNA complex at 5.4 Å resolution. Based on the information obtained from the structure and model building, a structural model for nucleoprotamine is proposed.

Materials and methods

Single crystals of transfer RNA were grown in a buffer containing 40 mM MgCl₂, 40 mM Na cacodylate, 3 mM spermine hydrochloride at pH 6.0 (ref. 10). Yeast phenylalanine tRNA was purchased from Boehringer-Mannheim, and protamine sulphate from salmon sperm was purchased from Sigma. Protamine sulphate was dissolved in the same buffer solution as the tRNA crystallisation buffer, and then added to a small depression containing tRNA crystals to yield a protamine-tRNA molar ratio of 4:1. After several days, the soaked crystals were sealed in glass capillaries and X-ray data were collected on an automatic four-circle diffractometer.

The sizes of crystals used for collection of X-ray data were about 100 × 150 × 500 μm. The soaked crystals had the same space group symmetry, and the cell dimensions changed less than 0.4%. Five steps per reflection were measured and individually fitted to a Gaussian curve to estimate integrated intensities for each reflection. A total of 1,253 reflections was collected up to 5.4 Å resolution, and of these, 1,011 had intensities significantly (greater than 4σ: where σ is an estimated standard deviation of each intensity) above the background. This data set was scaled to that obtained from a native tRNA crystal (which did not contain any protamine) as a function of resolution. The discrepancy factor (*R* factor) between these two data sets was 8% for the structure factor amplitudes. A difference electron density map was prepared, using the differences of amplitudes between these two data sets and the phases calculated from the atomic coordinates of the yeast tRNA^{Phe} structure in an orthorhombic lattice. The three-dimensional structure of this tRNA has been determined¹¹ and refined by a structure factor least-squares method¹².

Eleven residues from the C terminus of a salmon sperm protamine, Salmine A1, were folded to generate an α helix and then fitted to the difference electron density map obtained as described above, using an interactive computer graphics system at the University of North Carolina, Chapel Hill. After a portion of the α helix was fitted approximately into the electron density, it was further refined by treating the α helix as a rigid body. Three rotational and three translational parameters, as well as the occupancy and the overall temperature factor, of the α helix were refined by minimising the difference between calculated structure factors and the observed structure factors using a least-squares method¹³.

Circular dichroism (CD) measurements were made on a Dichrographe III of Jobin-Yvon using a quartz cell with path-length of 1 mm. The stock solutions for the CD measurements were: a buffer solution containing 10 mM MgCl₂, 10 mM Na cacodylate and 1 mM spermine·4HCl at pH 6.0; a tRNA solution containing 5.3 mg ml⁻¹ of yeast tRNA^{Phe} in the buffer described above; the protamine solution containing 12.5 mg ml⁻¹ salmon protamine sulphate in the same buffer. CD profiles were recorded for each of these solutions, and for

mixtures with protamine-tRNA molar ratios ranging from 0:1 to 16:1.

Structure of a protamine-tRNA complex

The results of the CD measurements are shown in Fig. 2. As protamine was added to the tRNA solution, we observed an increase of precipitation, and a decrease of tRNA in solution, indicated by the reduction in the magnitude of θ while the curve shape remained the same. When the molar ratio of protamine to tRNA reached 4:1, practically all tRNA and protamine in the solution was precipitated (Fig. 2*f*). This molar ratio corresponds to a stoichiometry of 1.1 positive charges of protamine per 1 negative charge of tRNA, if one assumes that salmon sperm protamine A1 is representative of all salmon sperm protamines. At present, this is the only protamine from salmon sperm that has been sequenced¹³, and it contains 32 residues of amino acids of the following sequence:

```
PRO ARG ARG ARG ARG SER SER ARG
PRO VAL ARG ARG ARG ARG ARG PRO
ARG VAL SER ARG ARG ARG ARG ARG
ARG GLY GLY ARG ARG ARG ARG
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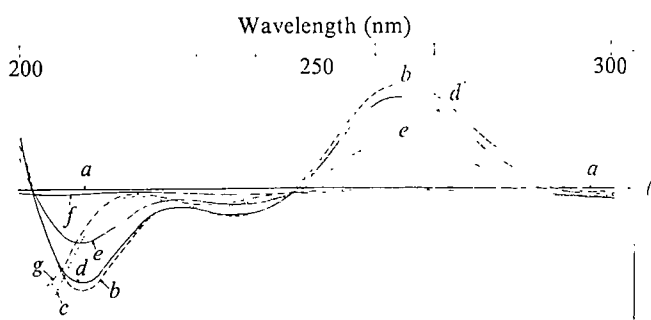


Fig. 2 Circular dichroism spectra of complex formation between salmon sperm protamine and yeast phenylalanine tRNA. The molar ellipticity θ is on one axis and the wavelength (nm) on the other. The θ value of curve *b* at 265 nm is 1.895×10^3 degrees cm² dmol⁻¹. The measurements were made for the wavelengths between 300 nm and 205 nm. *a*, A buffer solution containing 10 mM MgCl₂, 10 mM Na cacodylate and 1 mM spermine·4HCl with pH adjusted to 6.0; *b*, tRNA solution containing 1.0 mg ml⁻¹ of yeast tRNA^{Phe} in the buffer solution described above; *c*, the protamine solution containing 4.0 mg ml⁻¹ salmon protamine sulphate in the same buffer; *d*, 12.5 mg ml⁻¹ protamine stock solution was added to the tRNA solution described for *b* to give a molar ratio of protamine-tRNA of 0.25:1; *e*, molar ratio of 1:1; *f*, molar ratio of 4:1; *g*, molar ratio of 16:1.

The difference electron density map revealed only one kind of long, partially segmented electron density peak, running zig-zag along the small channel (channel *b* in Fig. 1) formed by tRNA molecules in crystalline lattice as shown in Fig. 3. This was the only peak present when contoured at the level of 5σ where σ represents root mean square electron densities in the difference electron density map. There were no significant negative peaks either. Each electron density segment is approximately cylindrical and corresponds in size to an α helix of about nine or ten amino acids (see Fig. 3*C*). Each segment is in contact, on a shallow (minor) groove side, with both strands of one double helix from a tRNA molecule (see Fig. 4) and one strand each of two other double helices from a neighbouring tRNA molecule.

When the α-helical portion of the structure was refined by a least-squares method, the occupancy and the thermal parameter were 20% and 90 Å² respectively. Since the asymmetric portion of the electron density can accommodate only about nine or ten of 32 amino acid residues, we assume that a part or all of the protamine molecule is partially disordered along the chain of electron density and that the kinking of the electron densities probably represents the kinking of the α helix at sites prior to

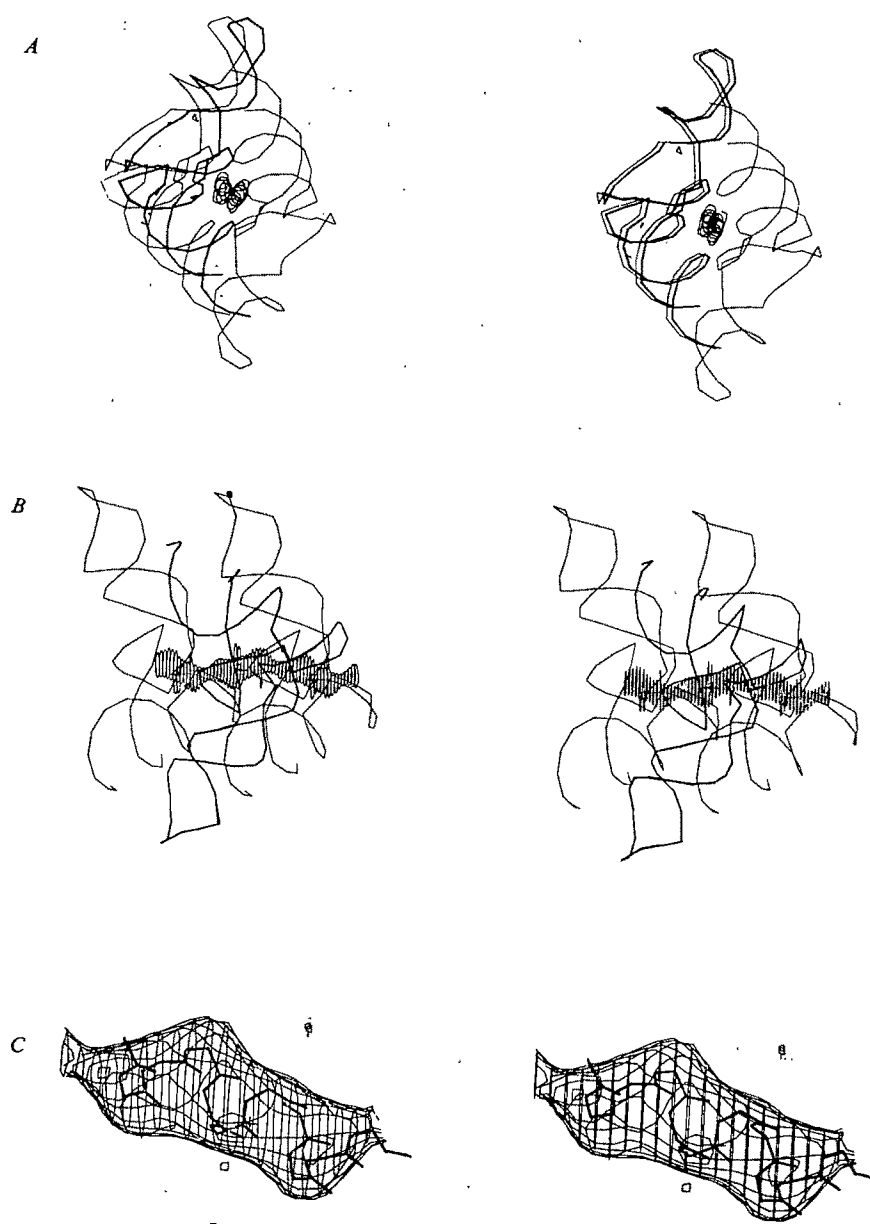


Fig. 3 The difference electron density map between the crystal of protamine-yeast tRNA1/1 and the native crystal of yeast tRNA1/1. The contouring level was chosen to show, primarily, the major feature of the difference electron density. The backbone structure of the tRNA is superimposed to show the relative position of the electron density peaks in the crystalline lattice. *A*, viewed approximately along the crystallographic axis *a* (for clarity, only two cylindrical segments are shown). *B*, Three cylindrical segments, viewed along the crystallographic axis *b*. The channels are running approximately along the crystallographic axis *a*. *C*, The fitting of a unique asymmetric portion of the difference electron density map with an α -helix ten residues long.

two internal prolines and a pair of glycines in the amino acid sequence (see Fig. 5 and below).

Conclusions and discussions

On the basis of our results from single crystal X-ray diffraction and CD studies, we make the following conclusions.

The protamine molecule changes its conformation from a random coil structure to a structure containing one or more α -helical segments in the presence of tRNA and possibly other nucleic acids. This is based on the observation that protamine in solution, in the absence of tRNA, gives a CD spectrum typical of a mostly random coil (see curve *c* of Fig. 2). This observation is consistent with the earlier observations from deuterium exchange and spectroscopic experiments^{4,14}. In the presence of tRNA, however, the protamine forms a complex with tRNA and precipitates. The crystal structure of the protamine-tRNA complex suggest that at least a portion of the protamine which is in contact with tRNA is in an α -helical conformation.

In a protamine-tRNA complex, the stoichiometry of positive charges of protamine to negative charges of tRNA is approximately 1:1. This is based on the observation from the CD study which shows that when the molar ratio of protamine to tRNA is 4:1, all the tRNA and protamine have been complexed and precipitated and no appreciable tRNA or protamine remains in

solution. At such a molar ratio, the stoichiometry between positive charge from protamine and negative charges from tRNA is approximately 1 to 1. This charge stoichiometry is the same as that observed for protamine-DNA complexes (for review, see ref. 8).

The protamine molecule binds to tRNA nonspecifically. As mentioned earlier, the yeast phenylalanine tRNA in the orthorhombic crystal form occupies only 25% of the crystal volume, with the remainder being buffer solution. tRNA molecules in this crystal form make a small number of contacts which barely maintain the crystalline lattice, thus most of the molecular surface of tRNA is exposed to buffer solution and should therefore be available for protamine binding. In fact, the absorbance measurement at 202 nm of the mother liquor in equilibrium with tRNA crystals showed that more than 99.3% of the protamine added was bound in the tRNA crystals. The difference electron density map, however, shows that the significant peaks are found only in the small channel *b* of Fig. 1. Since the single crystal X-ray diffraction studies can reveal only the ordered or partially ordered portion of the protamine structure, this crystallographic observation can be interpreted as follows: The protamine binds to all available parts of tRNA. But they bind with no site specificity and, therefore, they are not ordered or partially ordered in a crystalline lattice. In channel *b*, however, which is relatively narrow and restricted spatially by

the tRNA molecules nearby, the protamine molecules probably have much less freedom in binding and thus are partially ordered, and therefore 'visible' by single crystal X-ray diffraction techniques.

Protamine molecules, or the α -helical portion of protamine molecules, can stabilise not only an individual double helix, but also two or three adjacent double helices, thus helping the close packaging of double-helical RNA and possible DNA. This is based on the following observations: at a 4:1 molar ratio of protamine-tRNA, protamine helps to aggregate and precipitate tRNAs; in the crystal, the α -helical portion of protamine is sandwiched between a double-helical portion of tRNA from one molecule and one strand each from two more double-helical portions of a neighbouring tRNA molecule (Fig. 3A). Within this contact region, the distance between the α helix of the protamine segment and the phosphate groups of tRNA suggests that the guanidinium groups of extended arginine side chains can hydrogen bond to and, at the same time, neutralise, the negative charges of phosphates of tRNA. In the crystal, the α -helical segment was seen to bind to the shallow (minor) groove but not to the deep groove of the double helical portions of tRNA (Fig. 4), probably because only the shallow grooves of the T stem and acceptor stem are exposed to channel B.

From these conclusions, some general comments may be made about the possible mode of interaction between protamine and double-helical RNA or DNA. Given that protamine changes its conformation from a random coil to an α -helical structure in the presence of double-helical RNA or DNA, then Salmine AI protamine can be described, for example, as a structure with four segments of α helices with an average of about eight residues each, joined by three partially flexible joints, as shown schematically in Fig. 5. These joints are at residues 9, 16 and 26, all followed by well known helix-breaking residues, proline and glycine¹⁵. This prediction also follows from the consideration that, since each segment contains similar amino acids, if one segment assumes α -helical conformation in certain conditions, the others are also likely to assume the same conformations in the same condition, yielding four α -helical segments.

Nucleoprotamine model

The amino acid sequences of several fish sperm protamines have been determined (for a convenient compilation, see ref. 16). All of these sequences have the following aspects in common (see Fig. 6): they are 31–34 residues long; each begins with proline or alanine, followed by eight to ten residues, of which all except three are basic; and then another proline residue followed by five or six residues, all except one being basic; then another proline or tyrosine, followed by 13–17 residues,



Fig. 4 Schematic drawing showing the location of an α -helical segment bound to the shallow groove of the T stem of yeast phenylalanine tRNA. The α -helical segment is shown as a cylinder. The shallow grooves in the tRNA structure are shaded.

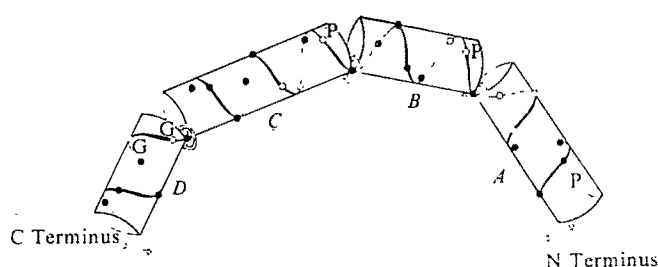


Fig. 5 Diagrammatic representation of Salmine AI protamine structure. This protamine is assumed to be composed of four α -helical segments joined by three partially flexible joints at residues just prior to two internal prolines and one joint before a pair of glycine residues. Arginines are shown as dark circles; and P and G stand for proline and glycine respectively.

all except three to six being basic amino acids. Thus, one can consider the general structure of protamine as composed of at least three segments, with two middle joints being a proline, tyrosine, or alanine residue. Even the last segment can be subdivided, in most cases, into two with a joint before a pair of glycines or valines.

Based on the observations from the crystal structure of the protamine-tRNA complex described here, we propose that all protamines have a structure composed of three or four α -helical domains connected by two or three flexible joints as suggested in Fig. 6 and shown schematically in Fig. 5 for Salmine AI protamine. Each α -helical domain contains four or more consecutive arginine residues, and can lie approximately along either groove of double-helical DNA. Two arginines facing the groove can neutralise and hydrogen bond to two negatively charged phosphates across a groove of one double helix. At the same time, the remaining arginines in the same α -helical domain can hydrogen bond to and neutralise the negatively charged phosphates of the neighbouring double helices, thus 'condensing' many double helical DNAs.

Although the backbone of an α helix does not have a structural complementarity to double-stranded nucleic acids as was noted for a β ribbon¹⁷, two nearby residues with long side chains like arginine and lysine in an α helix can place both basic groups about 10 Å–16 Å apart, depending on the side-chain conformation, just suitable to span across either groove of a double-stranded DNA or RNA.

A schematic drawing of the nucleoprotamine model is shown in Fig. 7 and described below. Model building suggests that the α -helical domains are more likely to lie on the major groove of double-helical DNA, although the minor groove cannot be ruled out. Two (or three when the arginine tract is longer than four) arginines of each domain neutralise and hydrogen bond to two (or three) phosphates across a major groove and the remaining arginines do the same to the phosphates of neighbouring double helix or helices. Each domain is thus binding or 'cross-linking' two or more double helices together, thereby causing a heavy condensation of DNA double helices.

Since the protamine α helices lie mostly on the major groove in this model, it is consistent with the observation in X-ray diffraction pattern of nucleoprotamine fibres, where the first layer-line intensities are stronger compared with the second layer-line^{1,2}. The di- or trivalent binding property of the α -helical segments of protamine can explain the electron microscopic observation on reconstituted nucleoprotamine⁶, where double-helical DNAs were shown to form a network. This also explains the observation that nucleoprotamine fibres do not swell as much as DNA on exposure to high humidity¹. Such cross-linking ability is likely to stabilise superhelical structure of DNA also. Although α -helical domains of protamine lie on the major groove of DNA double helix, the surface of the groove is essentially exposed to the solvent, because of the large spacing between the axis of the α helix and the groove surface caused by the long reach of arginine side chains (shown

SALMINE AI	P + + + + S S S +	P V + + + + +	P + V S + + + + +	G G + + + +
IRIDINE I(A)	P + + + + S S S +	P V + + + + +	P + + V S + + + + +	G G + + + +
IRIDINE I(B)	P + + + + + S S S +	P I + + + + +	P + + V S + + + + +	G G + + + +
IRIDINE II	P + + + + S S S +	P V + + + +	A + + V S + + + + +	G G + + + +
THYNNIN Y1	P + + + + E A S +	P V + + + + +	Y + + S T A A + + + + +	V V + + + +
THYNNIN Y2	P + + + + Q A S +	P V + + + + +	Y + + S T A A + + + + +	V V + + + +
THYNNIN Z1	P + + + + + S S +	P V + + + + +	Y + + S T V A + + + + +	V V + + + +
THYNNIN Z2	P + + + + + S S +	P V + + + + +	Y + + S T A A + + + + +	V V + + + +
CLUPINE Z	P + + + + S + + A S +	P V + + + +	P + + V S + + + +	A + + + +
CLUPINE YI	A + + + + S S S +	P I + + + +	P + + + T T + + + +	A G + + + +
CLUPINE YII	P + + + T + + A S +	P V + + + +	P + + V S + + + +	A + + + +

HELIX A
HELIX B
HELIX C
HELIX D

Fig. 6 All the known sequences of protamines¹⁶ are arranged to show four proposed domains. Abbreviations: A, alanine; E, glutamic acid; G, glycine; I, isoleucine; P, proline; Q, glutamine; S, serine; T, threonine; V, valine; Y, tyrosine; +, arginine.

schematically in the inset of Fig. 7). This aspect of the model is consistent with the results that dimethyl sulphate can methylate the N-7 atom of guanine in the major groove and the N-3 atom of adenine in the minor groove of the DNA double helix with equal relative efficiency but with small retardation on the major groove side⁷ at low temperature.

Functional implications of the model

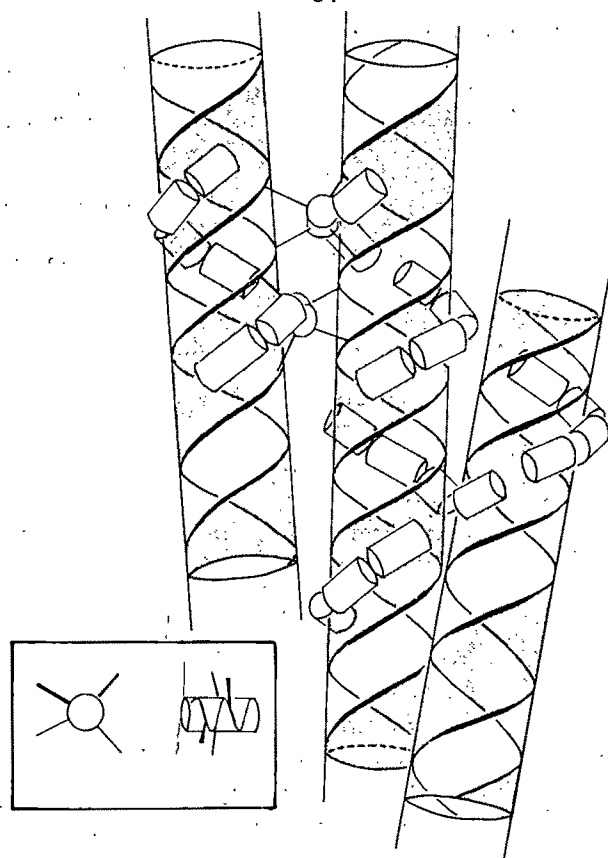
The protamine structure proposed here has several advantages as a DNA-condensing agent: The α -helical domains have structural rigidity and just sufficient length to stabilise DNA double helix-double helix cross-linking or 'condensation'; also, the joints between the α -helical domains provide flexibility in the orientation of the domains, so that two DNA double helices in variety of orientation can be brought together. Such extensive DNA condensation is probably the major reason for the inhibition of DNA-dependent RNA synthesis by protamines¹⁸.

The newly synthesised protamines are usually phosphorylated at serine residues¹⁹. The model proposed here suggests that such modification on protamines will interfere with the cross-linking of two adjacent DNA double helices. The following sequence of events can be imagined: The phosphorylation of protamines is probably necessary so that the modified protamines can efficiently displace histones and prevent DNA condensation (which will complicate the process). Once histone displacement is almost complete, dephosphorylation starts and so does the concomitant DNA condensation as a necessary step in the sperm maturation process.

The proposed model can explain several experimental observations and suggests some functional implications; however, the reason for the existence of multiple components in a protamine is not obvious from the model. It is likely that the similar interaction between two well defined structural elements, the α helix of protein and double helix of nucleic acids, may be found in other complexes between proteins and double-helical nucleic acids, such as in nucleosomes.

We thank Yasuhiko Nozaki, John Boyle, Joel Sussman, Steve Holbrook, and Charles Alden for help and discussions. This work was supported by grants from the National Institutes of Health and the National Science Foundation. The model fitting and some of the drawings were done with the aid of the GRIP-75 molecular graphics system, built by the University of North Carolina Department of Computer Science. Major system contributions were made by E. G. Britton, J. S. Lipscomb, M. E. Pique, W. Siddall, J. E. McQueen Jr,

Fig. 7 Schematic drawing to show parts of nucleoprotamine structure. Protamine molecules are represented by several connected cylinders; each corresponds to an α -helical segment. They wrap around the major groove (shaded) of the DNA double helix shown as a long large cylinder. Inset, an α -helical segment with four consecutive arginine residues (represented as four rods radiating out from the cylinder) is shown in two-orthogonal orientations. Two types of possible cross-linking are shown. Between the left and middle DNA double helices, there are two α -helical segments with arginine side chains shown as straight lines. Each segment forms two contacts across a major groove of one DNA double helix and two across a minor groove of the other DNA double helix. Between the middle and the right DNA double helices, three α -helical segments from one protamine molecule are in contact on the major groove of the middle DNA double helix and the fourth segment on the major groove of the right DNA double helix. There are many other types of cross-linking possible.



J. Hermans, W. V. Wright and F. P. Brooks Jr (principal investigator) and the system development was supported by NIH, NSF, AEC and IBM.

Received 26 August; accepted 1 November 1977.

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letters to nature

X-ray lines and magnetic field of Her X-1

THE report¹ of a possible detection of an X-ray emission line with energy about 53 keV from the binary X-ray source Her X-1 created great excitement. Interpreting the line as arising from non-relativistic electron cyclotron emission at the surface of an accreting neutron star member of the binary star system, Trumper *et al.*¹ deduce a neutron star surface magnetic field of 4.6×10^{12} G. If the line is real (of cosmic origin and not due to background effects), significant (not due to a statistical fluctuation) and correctly interpreted as arising from non-relativistic cyclotron emission) it could provide the first direct and quantitative measure of the magnetic field of a neutron star, and be direct support for our ideas on the formation of neutron stars and the role of magnetic fields in the radiation mechanisms of pulsars and binary X-ray sources. Furthermore, it would conclusively rule out the possibility that Her X-1 contains a rotating or pulsating white dwarf (which, for dynamical stability requires $B \leq G^{1/2} M R^{-2} \approx 10^{12}$ G for $M \approx M_{\odot}$ and $R = R_{WD} \approx 10^9$ cm).

Coe *et al.*² reported the detection of a line at 64 ± 6 keV in the Her X-1 X-ray spectrum from observations made with the Ariel 5 satellite. The energy of their line is consistent, within observational uncertainties, with the line energy reported by Trumper *et al.*¹ (53 ± 8 keV). In light of these two reports, we have re-examined the UCSD OSO-7 observations³ of Her X-1 made in 1971 and 1972. As can be seen in Fig. 1, these data from May to June 1972 contain a large deviation from the blackbody best fit at about 25 keV. This feature seems to be about as significant (or insignificant) as that reported by Coe *et al.*² No X-ray flux was detected at energies above about 50 keV for this source. The > 50 keV data were, therefore, combined to form the upper limit shown in Fig. 1. As can be seen, the best fit continuum falls steeply and we could set no meaningful upper limit to a 60 keV line.

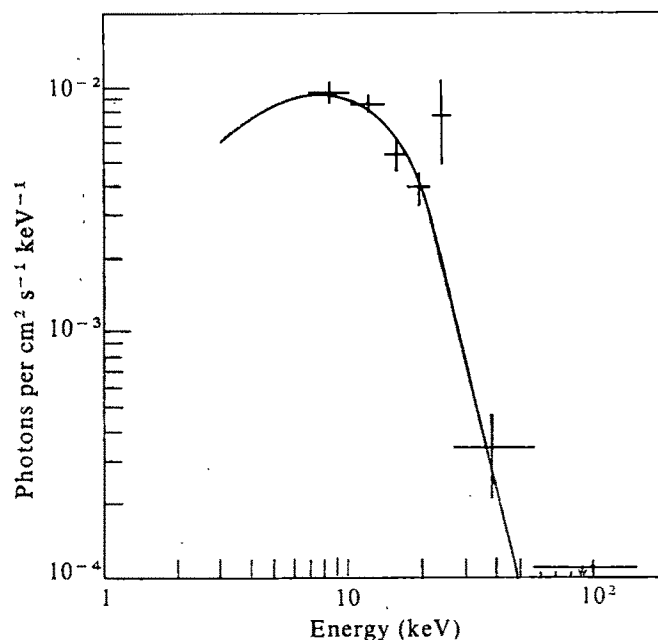
The observations of Her X-1 were made with the UCSD OSO-7 NaI (TI) 64 cm² 1 cm thick actively collimated detector. The spin rate of the satellite was 2 s per rev. This allowed continuous measurements of both the X-ray source and detector plus sky background^{3,4}. The 29 May to 1 June Her X-1 spectrum derived shows a feature at about 25 keV. If this feature were due to a line from Her X-1 itself, we estimate the intensity of the line as $2.4 \pm 1.2 \times 10^{-2}$ photons cm⁻² s⁻¹ centred at about 25 keV. A cosmic X-ray line would produce a deviation in at least one other energy channel (since one channel is about 4 keV wide), and by a judicious choice of continuum, a cosmic X-ray line is consistent with the data. But, there are alternative interpretations of such a feature. We discuss the two most likely ones below.

The UCSD OSO-7 instrument was subjected to bombardment by many low energy protons and neutrons during its passages

through the South Atlantic Anomaly. As a result, several features were present in the detector background, including an apparent line at about 30 keV, as well as a line at about 60 keV as shown in Fig. 2. These features are due to conversion of the iodine in the NaI (TI) crystal to radioactive nuclei. Effects such as this have been discussed extensively^{5–9}. In the UCSD OSO-7 experiment, the detector background 25 keV feature is comparable in intensity to the derived 25 keV flux from Her X-1. We tried to eliminate the feature from the Her X-1 data by using only the results taken a long time after the last South Atlantic Anomaly passage of the satellite. The feature remains as shown in Fig. 1. We estimate that an error in the background subtraction could have caused no more than a 10% error in the derived 25 keV flux from Her X-1. The one outstanding feature, however, in the OSO-7 Her X-1 data occurs at just the energy of one of the X-ray detector's background lines.

Trumper *et al.*¹ do not report a line at ~ 25 keV. If the OSO-7 Her X-1 feature were cosmic in origin, it might be variable, or Trumper *et al.* and Coe *et al.* might not have had the sensitivity to

Fig. 1 Average spectrum of Her X-1 from the UCSD OSO-7 cosmic X-ray detector³. Observations from 29 May–1 June 1972. The solid line fit is a minimum blackbody fit giving $T = 5.6 \times 10^7$ K.



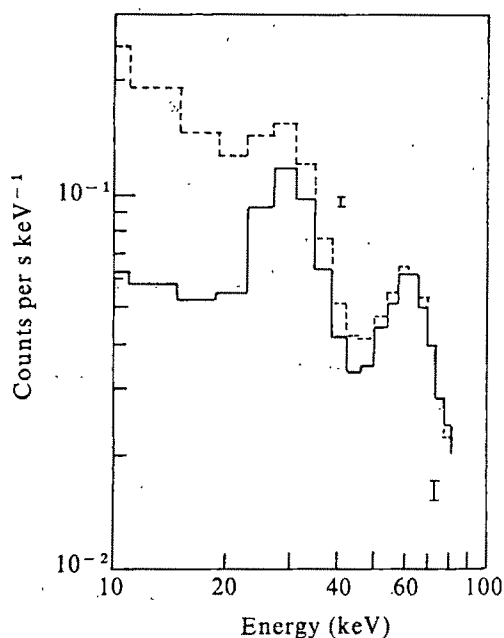


Fig. 2 Energy deposition spectrum for the UCSD OSO-7 cosmic X-ray detector. The solid line is derived from Earth-looking portions of the satellite orbit. The dashed line is derived from sky looking data excluding strong cosmic X-ray sources⁸.

an X-ray line of the OSO-7 instrument near 25 keV. The lowest energy that the Coe *et al.*² instrument could detect is about 25 keV, which might not have been low enough to be relevant to this report.

Another explanation that cannot be excluded is that the 25 keV Her X-1 feature is simply a statistical fluctuation. This is because: (1) the data point is only about 2σ above the continuum flux and (2) *a priori*, we do not know where to expect the line, and therefore finding a line in any one of five channels is five times as likely as deduced on the basis of simple Poisson statistics. This makes the present 'detection' only about 1.5σ . But, the same factor applied to the results of Trümper *et al.*¹ for example, also reduces the significance of the results from their two detectors from 6.2 and 4σ to about 5σ and 3σ respectively.

Adding the statistical uncertainty to the possibility of background contamination, it is unlikely that the UCSD OSO-7 keV feature in the Her X-1 spectrum is of cosmic origin. On the other hand, we cannot exclude such a possibility. We shall therefore briefly discuss the consequences of assuming the line is real and arises from cyclotron emission from Her X-1.

Even if one or all of the line features discussed above are of cosmic origin and arise from cyclotron radiation from electrons moving in the strong magnetic field of a neutron star, many uncertainties arise in trying to make a quantitative determination of the value of the B field. This is important because we already have rough arguments supporting the notion of 10^{12} – 10^{13} G magnetic fields at the surface of neutron stars (the value expected from collapse of a main sequence star of radius $R_0 = 10^{11}$ cm and surface magnetic field 100 G by conserving flux BR^2 to a neutron star of radius $R_n \approx 10^6$ cm). We summarise some of these estimates below⁹. (1) The naive theory of pulsar emission suggests that the pulsar energy loss rate is related to the pulsar radius R , magnetic field B , angular velocity Ω , spin down rate $\dot{\Omega}$ and moment of inertia I by

$$dE/dt \approx I\Omega\dot{\Omega} \approx B^2 R^6 \Omega^4 f(R\Omega/c)/c^3$$

where $f(R\Omega/c)$ is a dimensionless number, probably of order 1. From the observed values of dE/dt for the Crab Pulsar NP0532 and of Ω and $\dot{\Omega}$ for other pulsars one finds $B \approx 10^{12}$ – 10^{13} G. (2) Accretion models of binary X-ray sources such as Her X-1 suggest $B \approx 10^{12}$ to 10^{13} G and require $B > 10^{10}$ G in order for the Alfvén radius to be greater than the stellar radius. (3) Finally, for

dynamical stability of the neutron star, one requires $B \ll 10^{17}$ G.

Many factors play a part in making a quantitative derivation of the magnetic field strength from the observation of the X-ray line. For example, if one assumes that the field has a magnetic dipole configuration, the emission must arise¹ from a region with $\Delta B/B < 0.3$ so that the line is not broadened too greatly, in which case one has $\Delta r/r < 0.1$. Emission arises then from a region within 1 km of the neutron star surface. Second, the emitting region must be optically thin, so that the line is not broadened, shifted and smeared beyond recognition. The detailed radiative transfer in this region is not known, various theoretical models of such effects notwithstanding^{11–13}. Next, a line emitted from the surface of a neutron star must be corrected for redshift¹⁴ which can range from ~ 0 to as much as ~ 0.6 . (This depends on the mass of the star, its equation of state, and the theory of gravity used. For incompressible matter in general relativity, however, $z_{\max} = 2$.) Fourth, if the magnetic field is of order 10^{12} G or greater, the classical electron cyclotron radiation energy will be $\Delta E = \hbar\omega = (e\hbar/mc)B$ for electrons of $v/c \ll 1$. But for $B \approx 10^{12}$ G, $\Delta E \sim 50$ keV, the energy is not negligible compared to the electron rest mass energy. Looked at another way², as the de Broglie wavelength for a 50 keV electron is greater than its cyclotron radius, a relativistic quantum mechanical treatment of the problem is necessary. For electrons with momentum p_{\parallel} parallel to the field, spin state s and principle quantum number n , the energy levels found as eigenvalue solutions to the Dirac equation are given by

$$E = \pm [c^2 p_{\parallel}^2 + m_0^2 c^4 + e\hbar c B(2n + s + 1)]^{1/2}$$

Thus depending on what spin or principle quantum numbers are chosen, the deduced magnetic field will differ from that derived using a non-relativistic (classical or quantum) treatment. For example, if one considers only n states (with $s = -1$, say) the relativistic and non-relativistic treatments can give results differing by a factor of ~ 2 . If one takes all of these effects into account, the deduced field strength can vary from 10^{12} G (for a neutron star of mass $M \approx 0.6M_{\odot}$, lowest energy level or first harmonic at 25 keV) to about 10^{13} G (for a neutron star of $2M_{\odot}$ and taking the first harmonic as the 64 keV line). It is comforting that this range covers the field range deduced from other arguments. A more precise result would require much more detailed information about the neutron star itself, the accretion rate, the topology of the B field, temperature of the radiating electrons, and a host of other parameters.

Observations in the near future from HEAO 1 should be able to establish the reality, energy pulse fraction and relative strength of one or both of the X-ray line features we discussed here. Perhaps detection of linear or circular polarisation in the lines could test the cyclotron origin. The discovery of lines at similar (but not exactly the same) energy might be expected from other X-ray binary systems containing accreting neutron stars.

K.B. is supported in part by the USNSF under grant No. AST74-19213 A02.

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Received 6 September; accepted 3 November 1977.

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Radioastronomical frequency for interstellar NH^+

SOME spectroscopic frequencies may be calculated more accurately than laboratory experiments can measure them. *Ab initio* molecular orbital calculations can be used to compute molecular properties, but none as accurately as Λ -doubling. This effect gives rise to splittings of molecular energy levels of diatomic molecules and transitions between the Λ -doublets are the sources of some of the most important radioastronomical spectral lines. Here we consider the as yet unobserved but very likely instellar species NH^+ .

Very crudely, Λ -doubling may be thought of as an effect of electron slip as the molecule rotates; the electrons lagging somewhat behind the rotating nuclear frame. This results in the energies of degenerate Π molecular energy levels becoming different, with an energy separation which increases as the rotational quantum number increases. Formally the effect may be described as the influence of Σ electronic states on Π states. The interaction, which is zero to a first approximation, is dominated by spin-orbit coupling, giving non-zero matrix elements between Σ and Π states.

The well-known astrophysical source, OH, was the subject of calculations¹ which would now be thought of as relatively crude, but in wave numbers the agreement with experiment is impressive (observed 0.0788 cm^{-1} ; calculated 0.0810 cm^{-1}). This encouraging result was, in a sense, fortuitous because the origin of the splitting in OH is particularly simple, being an example of Van Vleck's approximation of pure precession where the $^2\Pi$ electronic ground state is only seriously influenced by a single $^2\Sigma^+$ state.

The case of CH is more complex as several states of $^2\Sigma^+$ and $^2\Sigma^-$ symmetry have to be considered, but agreement with experiment³⁻⁵ is close enough for the computations to be within a few MHz of the observed astrophysical frequency.

We have now extended this work to the species, NH^+ , where a further complication enters the calculations. In this case we have to consider not only interacting $^2\Sigma^+$ states but also $^4\Sigma^-$ states. Configuration interaction wave functions and potential energy curves were calculated for the $X^2\Pi$, $a^4\Sigma^-$, $A^2\Sigma^-$ and $C^2\Sigma^+$ states of NH^+ using the Alchemy system of computer programs⁶. Using these wave functions and potential energy curves the interaction between the $X^2\Pi$ state and the $a^4\Sigma^-$ state was treated numerically, whilst the interaction with the $^2\Sigma^+$ states was calculated using second-order perturbation theory. It is important that because of the influence of the $^4\Sigma^-$ state, the Λ -doubling in the $X^2\Pi$ state of NH^+ cannot be interpreted in terms of the conventional constants p and q and each rotational level must be treated separately.

The results of these calculations predict Λ -doubling in the lowest rotational level of $^{14}\text{NH}^+$ to be 13.625 MHz. This may be compared with the best laboratory experimental value of $13.520 \pm 300\text{ MHz}$ for which special measurements were made⁷.

The splittings to be expected in $^{14}\text{ND}^+$ and $^{15}\text{NH}^+$ together with values in excited rotational levels for which accurate experimental measurements are not available will be published elsewhere with fuller details of this work.

Our predicted frequency of NH^+ is within the range of available radiotelescopes and the calculations could provide the first example of a case where theory has predicted a radio source rather than merely confirmed its identification.

The computational work has been supported by a grant from the SRC and forms part of a project supported by a NATO research grant.

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Received 3 October; accepted 25 November 1977.

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Irreversibilities in the mechanism of photoelectrolysis

PHOTODECOMPOSITION of water using semiconducting electrodes is being widely investigated. Using a combination of theoretical methods and experimental results we have analysed the sequential processes involved in this phenomenon and show here that several steps are irreversible. Some of the consequences of these irreversibilities are discussed. A theory of electronic charge transfer between electrodes and aqueous electrolytes, based on an equilibrium approximation by Marcus¹, seems to be valid for metal electrodes. Gerischer² has considered both metal and semiconductor electrodes and concluded that in general the electron is isoenergetically injected (tunnels) from the occupied band state of the electrode into an unoccupied state of the electrolyte. Rearrangement of the molecular environment then occurs, irreversibly changing the energy of the electronic state of the electrolyte. Finally, reverse tunnelling from the relaxed state back into the electrode may occur.

The two states of occupancy in the electrolyte constitute a redox couple. For metals the forward and reverse currents become equal as equilibration occurs; thus, the Fermi level of the metal adjusts to the redox potential of the redox couple of the electrolyte. For semiconductors, electronic charge transport involving different bands (electrons in the conduction band and positive holes in the valence band) have been separately considered³. This situation can lead to either an equilibrium or a non-equilibrium charge transfer process between semiconductors and electrolyte. The equilibrium approximation frequently appears in analyses of electronic charge transport between semiconductors and electrolytes. In particular, Gerischer³ has invoked the quasi-Fermi level concept to describe the charge carrier concentrations at the semiconductor-electrolyte interface under illumination.

Here we point out some irreversible aspects of charge transfer across semiconductor-electrolyte interfaces for photogenerated minority carriers, and emphasise the importance of these irreversibilities in explaining photoelectrolysis⁴⁻⁷ and the mechanism of the operation of the photochemical diode⁸. Furthermore, we believe that as a consequence of these irreversibilities, the quasi-Fermi level concept and the associated thermodynamic arguments are of limited validity for high efficiency photoelectrolysis. Irreversibility, has previously been proposed to explain reduction of ferricyanide at a ZnO semiconductor electrode⁹.

The energy level diagram for the n -type semiconductor electrode-aqueous electrolyte interface involved in photoelectrolysis is shown in Fig. 1. The essential features of the semiconductor-electrolyte junction are from Nozik¹⁰; the electronic states of water are from Williams *et al.*¹¹. The bending of the conduction and valence band edges, E_c and E_v respectively, are determined by the space charge arising

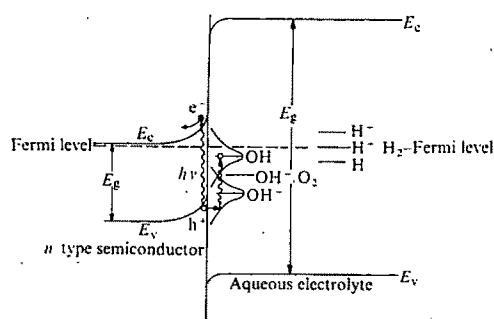


Fig. 1 Energy level diagram of *n*-type semiconductor (TiO_2 , $E_g = 3\text{eV}$) in contact with aqueous electrolyte ($E_g \approx 9\text{eV}$) showing energy bands of water and its extrinsic states. The energy levels of the extrinsic states have a distribution due to thermal fluctuations in the solvation structure; this is shown for the OH° and OH^- states.

from the difference in work functions of the semiconductor and of the electrolyte. The extrinsic states, H^+ and OH^- , are approximate, having been estimated from continuum theory¹¹. At the interface the macroscopic dielectric constant and the continuum approximation for small ions in solution are not strictly valid. The rearrangement energies can be similarly estimated, but for our purposes are taken from Henglein¹².

The sequence of events at the *n*-type semiconductor anode during photoelectrolysis are: (1) the incident quantum of radiation, $h\nu$, is absorbed in the semiconductor and thus creates a conduction electron and a valence band hole; (2) the electron, which is the majority carrier with energy very near the Fermi level, is displaced by the band bending into the external circuit, and the positive hole which is the minority carrier, and as such has energy available for radiative transitions or chemical changes, is displaced by the band bending towards the interface with the aqueous electrolyte; (3) the positive hole tunnels from the valence band edge into OH^- molecular ions at or near the interface; (4) a neutral OH° species is formed which causes the orientated H_2O molecules which surround the OH^- ion to relax, perturbing this extrinsic electronic state closer to the Fermi level; and finally (5) the chemistry to form O_2 proceeds according to the overall reaction: $4\text{OH}^\circ \rightarrow 2\text{H}_2\text{O} + \text{O}_2$.

Experimental data on the photoelectrolysis of water using *n*- TiO_2 anodes and Pt cathodes indicate that the quantum efficiency (that is, the fraction of absorbed photons producing charge carriers which are injected into the electrolyte to propagate the anode reaction) is high, especially if the band bending in the space charge region is reasonably large and the depletion width (w) is greater than the absorption depth ($1/\alpha$), where α is the absorption coefficient in cm^{-1} . Thus, for example, at 3,100 Å, where $\alpha = 6 \times 10^5 \text{ cm}^{-1}$ for TiO_2 , the quantum efficiency for the oxidation of water to O_2 at the TiO_2 anode is reported to be 1.0 (A.J.N. and R. R. Chance, unpublished and ref. 7). Under these conditions, the band bending is 0.9 eV (ref. 7), the absorption depth is 170 Å, and the depletion width is calculated to be 200 Å; the latter calculation is based on a carrier density of $2 \times 10^{19} \text{ cm}^{-3}$, which is typical for reduced TiO_2 crystals. At longer wavelengths, where the absorption depth increases, the quantum efficiency decreases. Thus, at 3,484 Å, where $\alpha = 1.1 \times 10^5 \text{ cm}^{-1}$ and the absorption depth is 910 Å, the quantum efficiency was measured to be 0.7 with a band bending of 0.9 eV. At this wavelength, the quantum efficiency decreases to values of 0.6, 0.5, and 0.2 at band bending values of 0.7 eV, 0.5 eV, and 0.3 eV, respectively.

The kinetics of photoelectronic injection from a semiconductor into an aqueous electrolyte depend on five time constants: τ_{recomb} , the time constant characterising the recombination rate of photogenerated electrons and holes; τ_t , the tunnelling time for charge transfer from the band

states of the semiconductor to the localised extrinsic states of the aqueous electrolyte; τ_r , the relaxation or rearrangement time characteristic of the extrinsic states of the aqueous electrolyte in going from the equilibrium condition of polarisation with the state unoccupied to the equilibrium condition with state occupied; τ'_t , the tunnelling time for charge transport from the relaxed occupied electrolyte state back into the semiconductor bands; and τ_c , the time constant for the occupied electrolyte state to undergo annihilation by chemical changes. Depending on the relative magnitudes of these time constants, various steps in the charge transfer may approach either equilibrium or irreversible conditions.

Under conditions of high quantum efficiency (0.8–1.0) as discussed above, the time constants are constrained as follows: $\tau_{\text{recomb}} > \tau_t$, $\tau'_t > \tau_t$, and $\tau'_t > \tau_c$. In other words, each photogenerated minority carrier must survive each step without appreciable probability of recombination with majority carriers, nor return to the semiconductor after injection. The latter condition is consistent with the low exchange currents observed with semiconductor electrodes¹³ and is understandable from the relaxation resulting in the occupied electrolyte state lying within the band gap of the semiconductor (see Fig. 1). The former condition is consistent with estimated values of τ_t and τ_{recomb} . The maximum tunnelling time, τ_t , can be estimated using the standard model of tunnelling¹⁴ from a potential well having a width of 200 Å (depletion width), a barrier height of 3 eV (difference between valence band edge of the semiconductor and that of intrinsic water), and a barrier thickness of 10 Å (distance of extrinsic OH^- levels from semiconductor surface). This thickness is greater than that of adsorbed OH^- ions, but less than the average inter-anion distance in the bulk electrolyte; the tunnelling rate is insensitive to the barrier thickness up to about 30 Å. In this model, carriers which have not undergone full intraband relaxation in the depletion layer ('hot' carriers) may also be injected into the electrolyte. The importance of band bending here is to confine the carriers within w , which increases the tunnelling rate. This calculation yields a value of about $5 \times 10^{-13} \text{ s}$ for τ_t . This is much faster than the estimated value of 10^{-10} s for τ_{recomb} which is calculated for bulk recombination in a direct band gap semiconductor with a majority carrier density of $2 \times 10^{19} \text{ cm}^{-3}$ (ref. 15). Surface recombination rates are more difficult to estimate.

Even faster tunnelling times would be obtained if the OH^- ion is taken to be closer to the semiconductor surface than 10 Å. For such strongly adsorbed molecular ions, a model involving a combined semiconductor-molecular ion eigenstate may be considered; this permits direct photoinjection of the hole to the OH^- ion without the necessity of a tunnelling process.

The concept of quasi-Fermi levels has been widely used in solid state physics in describing photoconductive, photovoltaic and semiconductor devices. The quasi-Fermi levels are different for electrons and for holes and describe the separate occupational probabilities for these two types of electronic particles among their respective band and defect states. The concept depends on local statistical detailed balance between the rates of creation and of annihilation of electrons and holes. For photoelectrolysis with high quantum efficiency, as noted above, local statistical detailed balance of electrons and holes is not achieved in the semiconductor near the electrolyte interface because the minority carriers are so rapidly depleted by tunnelling into the electrolyte; that is $\tau_{\text{recomb}} > \tau_t$. Therefore, the charge transfer process is better approximated as individual events. With the reverse tunnelling (exchange current) negligible, that is, $\tau'_t > \tau_t$, the charge transfer corresponds to irreversible photoinjection. This is similar, as regards irreversibility, to photoelectric emission into a vacuum; but the origin of the irreversibility is, of course, quite different.

In Fig. 1, the Fermi level of the semiconductor is shown above that of the OH^-/O_2 redox level, and it is equal to the Fermi level of the electrolyte. As O_2 is evolved at the semiconductor anode under illumination, and the concentration of OH° and other intermediate species accumulate, the reaction at the semiconductor-electrolyte interface will tend to equilibrate with the OH^-/O_2 redox level. This equilibrium will occur if the chemical depletion of OH° is slow compared to the relaxation time of OH° to its equilibrium state in the electrolyte, that is, if $\tau_0 > \tau_r$. With finite activation energy this condition is true, so that the anodic reaction in the electrolyte proceeds in approximate equilibrium with the OH^-/O_2 redox level; the absolute value of τ_r is 10^{-11} (ref. 16).

A new and simple device for the photochemical conversion of optical energy has recently been announced⁸. These devices have been labelled 'photochemical diodes', and consist of monolithic structures comprising either a semiconductor and a metal region or two different semiconductor regions (one *n*-type, the other *p*-type) which are connected through ohmic contacts. The latter is called a *p-n* heterotype photochemical diode, and it can exhibit voltage enhancement when the electron affinity of the *n*-type semiconductor is greater than the electron affinity of the *p*-type semiconductor⁹.

In accordance with the above discussion, the *p-n* photochemical diode can be described as a photocathode and photoanode, back-to-back, respectively irreversibly photo-injecting electrons and positive holes as minority carriers into extrinsic states of the electrolyte and with the majority carriers recombining in the ohmic contacts.

We thank Professor H. Gerischer for helpful comments, David Berry for independent estimates of tunnelling probabilities, and J. J. Gilman for support.

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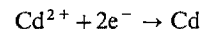
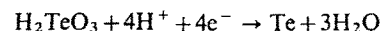
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Received 20 July; accepted 11 November 1977.

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The films of CdTe were prepared by the simultaneous electrodeposition of Cd and Te on a Ti base. Presumably the process is similar to that for CdSe film formation and involves the following reactions at the cathode:



The CdTe films were prepared galvanostatically in a two compartment cell in which 2 M H_2SO_4 was used as the anolyte, a solution of 0.01 M H_2TeO_3 , 1.5 M H_2SO_4 and 0.27 M CdSO_4 as the catholyte, platinum as the anode and titanium as the cathode. The compartments were connected by a KCl-agar salt bridge.

Before use the titanium was prepared by lightly sanding the surface to remove any oxide layer and then washed in hexane to degrease the surface. The best films were prepared from a stirred solution with the composition given above and using a current density in the range 30-40 mA cm^{-2} . The films formed were black and seemed uniform in texture and thickness, an upper limit for which was 8 μm . The films adhered well to the titanium. Heat treatment at 250 °C for 16 h increased the photovoltage obtained in the photoelectrochemical cell and also reduced its series resistance.

The films were tested in a nitrogen-purged electrolyte containing 1 M NaOH, 1 M Na_2S and 1 M S. The open-circuit voltage was 0.34 V, the short-circuit current 2.6 mA cm^{-2} and the fill factor 25%. The best power conversion efficiency obtained so far is 0.4% under an irradiance of 50 mW cm^{-2} of white light from a xenon lamp with a Schott KG4 filter.

When argon-purged 5 M NaOH, containing 0.03 M partly oxidised sodium telluride, was used instead of the sulphide-sulphur solution, the photovoltage at open-current was 0.29 V, the short-circuit current density, 2.2 mA cm^{-2} and the fill factor 27%. The power conversion efficiency for the same light as before was 0.4% when the irradiance was taken as that incident on the solution. Because the solution absorbed light, the power conversion efficiency with regard to light incident on the CdTe film was between 2 and 4%. Efficiencies can no doubt be improved by varying the deposition conditions and by using cells in which there is less absorption by the solution.

The Australian Research Grants Committee is thanked for support. An Australian provisional patent application PD2052 has been lodged pertaining to this technique.

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Received 12 October; accepted 3 November 1977.

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Photoelectrochemical cell with cadmium telluride film

ELLIS *et al.*¹ reported studies of single crystal CdX based photoelectrochemical cells when X = S, Se or Te and found that the best efficiencies were obtained when CdTe was used as the photoelectrode. Photoelectrochemical cells using thin films of CdS and CdSe have been reported by Miller and Heller² and by Hodes *et al.*³ In this paper, we report on a photoelectrochemical cell using a thin polycrystalline film of CdTe.

'Low scatter' conduction of electric charges through lamellar solids

MATHEMATICAL theories about the conduction of electricity through solids have been detailed for conventional metals¹. It is generally necessary to combine assumptions about the concentration of charge carriers with calculations about their probabilities of being scattered during flow. For synthetic metals the predominant scattering processes may be quite different, however. This may be so in lamellar solids after they have been converted into good electrical conductors by intercalation of electron donor or electron acceptor molecules between the layers. Such molecules are thought to form charge

transfer bonds with the two-dimensional macromolecules that constitute the layers in the parent solids before intercalation. Even static aspects of probable charge transfer bond formation have not always received complete quantum mechanical formulation. Dynamic aspects (which may influence scattering processes during current flow) are even less completely described. This letter points out that scatter probabilities of current carriers injected by charge transfer may be markedly lower than in the more familiar 'natural' three-dimensional metals. This may help to explain high electrical conductivities of lamellar synthetic metals.

Following an original hypothesis for lamellar compounds between graphite and a typical electron donor partner such as potassium, or a typical electron acceptor partner such as molecular bromine³, the remarkable increases of electrical conductivity, observed on forming intercalation compounds starting with crystalline layer lattices, were attributed to consequences of charge transfer bond formation between the giant planar carbon networks and electron donor or electron acceptor partners. Such resonance bonding may be comparatively weak, but until recently the fractional charge transfer f between partners has been only indirectly estimated. In alkali metal compounds with graphite³, just as with smaller aromatic molecules^{4,5}, volume occupancy calculations suggested that fractional charge f transfer must be near unity, but with electron acceptor partners a considerably smaller transfer seemed likely. These estimates were, however, confused by similarities in behaviour of synthetic metals⁶ such as dilute graphite-bromine, formed by direct intercalation of the halogen molecules, when compared with lamellar compounds such as graphite bisulphate formed by electrochemical oxidation⁷. In these electrochemical compounds the total quantity of electricity abstracted per g atom of graphite oxidised is accurately known. Plausibly this gives a direct measure of charge transfer fraction for electrochemical compounds. If valid, such an argument would be helpful by analogy, as electrochemical compounds are known in considerable variety⁸.

Recent optical studies⁹ give direct evidence, however, that in a lamellar compound, such as graphite-bromine, fractional charge transfer does not exceed a few tenths of 1% as most of the molecules of Br₂ are 'un-ionised'. This finding has vital consequences when interpreting the large increases of electrical conductivity (in the direction of the layer planes) on forming these synthetic metals. The structure and electrical properties of bromine-graphite are well known. A steep increase of conductivity is observed on intercalation of only very small mole fractions of Br₂ (ref. 6). The character of such increases is general for many kinds of intercalates, which at first (in dilute synthetic metals) occupy only a small fraction of the layers in the lamellar solid. Conductivity increases should apparently not be attributed specifically to any very large increase in the number of charge carriers made available per atom of carbon in the giant network conductors. To account for the high electrical conductivity of lamellar synthetic metals, the essential assumption seems to be¹⁰ that the comparatively few additional charge carriers transferred experience quite exceptionally 'low scatter' in the directions of the layer planes. At the present stage of research it might be misleading to term these lamellar solids 'super'-conductors. Cooperative effects between charge carriers are not excluded, but 'low scatter' describes the effects observed sufficiently.

The preceding remarks have been prompted by the considerable body of information on layer intercalates in graphite¹¹; however, 'low scatter' mechanisms of electrical conduction may be identifiable in other two-dimensional conductors, such as other lamellar intercalates. Even for one-dimensional, 'tunnel' or fast ion conductors scattering mechanisms seem likely to be much less effective than in conventional metals which are at least approximately three-dimensional. Whatever the nature of the solid framework in which localised charge transfer takes place, it may be important that the mobility of the molecular units from which transfer occurs remains high. They can then

participate in processes of momentum and energy exchange, together with the lighter charge carriers, when an applied voltage gradient leads to a drift of electric charge.

'Low scatter electrical conduction' parallel to the network has already been suggested by several highly unusual voltaic effects in lamellar compounds of graphite⁶. In particular, the Remote Voltaic Effect could (in one interpretation) be attributed to beams of charge carriers travelling in the lamellar solid but with each beam largely confined between its neighbouring pair of giant carbon networks; a physical analogy would be with a beam of light travelling down a curved film of glass, and unable to escape because of its total internal reflection at the air-glass surface. Unlike the transparent glass, in the lamellar compounds of graphite some general scattering seems unavoidable. Remote voltaic effects have, nevertheless, been detected over distances of several centimetres. By rigorous selection of the parent graphite specimens to be as near perfect as possible before intercalation, all these novel voltaic effects are generally more prominent and thus easier to observe. In addition to using selection criteria previously described, it is desirable to choose parent materials for which the magneto-resistance coefficient is high¹², and for which the intercalation threshold is very low¹³. Though methods for doing this are less well known, the importance of achieving low scatter by minimising structural imperfections probably also applies to diverse synthetic metals other than those based on graphite.

Study of all these effects has been greatly eased by the construction of a novel pulse meter¹⁴. This readily permits voltage gradient measurements using very large current densities up to 40,000 A cm⁻². Remote voltaic effects and other voltaic anomalies in lamellar conductors can be more readily followed with the much larger pulse currents that can now be used. The search for critical or threshold parameters is now also facilitated. Anomalous effects are not observed in the parent graphite despite its pronounced lamellar structure, but when the layers become separated by intercalation using diverse partner molecules, the marked increase in electrical conductivity and the appearance of unusual voltaic effects go side by side.

Even mass movement of the molecules themselves, acting as charge transfer partners, occurs with remarkable ease within the confinement of any intercalated layer. Some evidence^{10,11} indicates that the 'ripple potential' affecting such predominantly two-dimensional movement of molecules in a layer lattice seems far less restrictive than in most cases of mass diffusion in three-dimensional lattices and even compared with the extreme case of solid ionic good conductors (the so-called 'solid electrolytes').

Confinement to two-dimensional layers or even greater 'tunnel' restrictions on movement seems to affect markedly the electric charge carriers. Present data suggest that the unusual voltaic effects as well as the high electrical conductivity of lamellar synthetic metals are mainly due to 'low scatter conduction' of these charge carriers in the directions of the layer planes in graphite intercalates.

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Received 21 July; accepted 9 November 1977.

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X-ray photoelectron spectroscopic evidence for trapped oxygen species in irradiated NaClO_3

X-RAY Photoelectron Spectroscopy (XPS) has many advantages for studying, *in situ*, the photo-induced decomposition of inorganic molecular ions; this is shown by our data on the radiolysis of NaClO_3 , sodium chlorate, using the standard Mg ($K\alpha$) anode [1,254 eV, $\lambda \approx 10\text{\AA}$] in our AEI ES200B photoelectron spectrometer. We have already examined¹ the decomposition kinetics of NaClO_4 and NaClO_3 , when the final products of irradiation were found to be a defect NaCl phase together with molecular oxygen (detected by mass spectrometry). We report here some further low temperature XPS studies on the NaClO_3 system, where strong evidence for the presence of trapped oxygen species in the initial stages of irradiation has emerged.

Powdered samples were examined in the temperature range 83–293 K using a liquid-nitrogen cooled sample-probe coupled with a resistance heater and null-point thermistor giving automatic control within ± 5 K. A base pressure of 4×10^{-10} torr in the sample chamber was achieved by previous baking of the vacuum system. An MAT AMP3 quadrupole mass analyser was connected directly to the sample chamber, and pressures were measured with a cold cathode Penning gauge situated ~ 10 cm from the sample. At any given temperature the photoelectron spectrum of an NaClO_3 sample was recorded at various times of exposure to the exciting X radiation. The core-electron levels from chlorine [Cl(2p)] and oxygen [O(1s)] were of most interest although we also routinely recorded the Na(2s), C(1s), Cl(2s) and some sodium Auger peaks as calibration checks.

The rate of decay of the original Cl(2p) and O(1s) photoelectron peaks followed 1st-order behaviour, confirming our earlier results at 253 K¹. At temperatures in the range 113–233 K the O(1s) region developed an additional peak at ~ 5 eV lower kinetic energy (higher binding energy), which grew to a limiting value with X-ray dose before decaying slowly (see Fig. 1a and 1b). The original O(1s) peak was also observed to broaden with X-ray dose as its intensity declined. A sample of irradiated NaClO_3 with an appreciable low kinetic energy O(1s) component was allowed

Fig. 1 a, Lower trace: initial O(1s) photoelectron spectrum of NaClO_3 at 203 K; upper trace: same region after ~ 180 m irradiation. b, Intensity of low kinetic energy O(1s) peak as a function of $t_{1/2}$ (obtained from Cl(2p) intensity data).

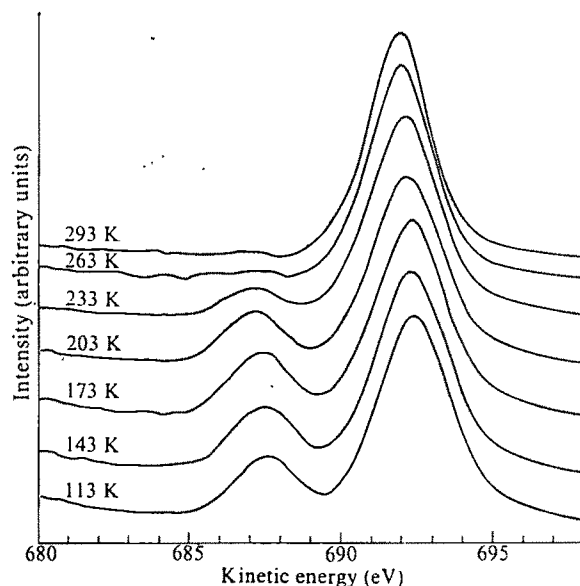
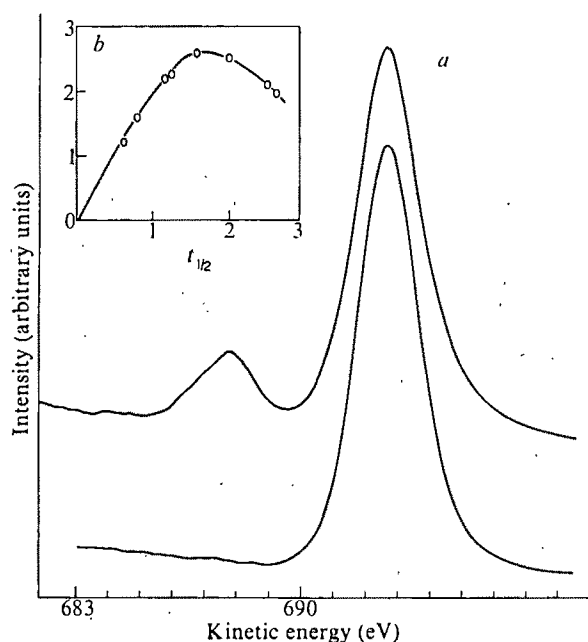


Fig. 2 Effect of raising the temperature on the O(1s) photoelectron spectrum of a sample of irradiated NaClO_3 , originally at 113 K. Sample chamber pressures were used to ensure equilibrium at each temperature.

to warm gradually to 293 K (see Fig. 2), when this feature was seen to decrease slowly, with a concomitant increase in the sample chamber pressure, due to $\text{O}_2(\text{g})$, (confirmed by intense peaks at $m/e = 32$ and 16 in the mass spectrum of the sample chamber atmosphere).

For irradiation periods much larger than the half-life of ClO_3^- , (when the Cl(2p) signal associated with the molecular ion had virtually disappeared), the main O(1s) signal remained roughly constant at $\sim 20\%$ of its initial value. A consistent interpretation of these results can only be obtained if the smaller O(1s) component is assigned to an oxygen species incorporated by the crystal lattice. The possibility that it is due to a weakly-bound oxygen molecule adsorbed on the surface was ruled out by studying the O(1s) regions of other ionic crystals which are chemically stable to ionising radiation (NaCl , Na_2SO_4). The only peaks observed had binding energies similar to the main O(1s) component in NaClO_3 ; thus the smaller O(1s) component arises solely from the induced decomposition of the molecular ion.

An electron spin resonance (ESR) study² on irradiated KClO_3 postulated the formation of molecular anions O_2^- ; which can be accommodated at vacant anion sites, providing an electron trap equivalent to the F-centre in NaCl. They exist in appreciable concentrations and show a fair degree of thermal stability. The low-intensity O(1s) component cannot be assigned to this species, however, since its intensity decreases as the decomposition of NaClO_3 proceeds, and is also very sensitive to the sample temperature. We have, therefore, assigned the residual component of the main O(1s) peak to O_2^- with a binding energy similar to that reported for oxides and chemisorbed oxygen species³.

The second low-intensity component at higher binding energy implies a small negative charge per atom. The average volume of the interstitial site in NaClO_3 (ref. 4) is $\sim 15\text{\AA}^3$ which is large enough to accommodate an oxygen atom of $\sim 12\text{\AA}^3$ volume, (calculated from the van der Waals radius). Similar considerations for the more closely-packed NaCl ⁵ structure yield an interstitial cavity of only $\sim 8\text{\AA}^3$. We cannot assign the low intensity component simply to an interstitial oxygen atom, however, because the multiplet splitting of the O(1s) level corresponding to the $^2\text{P}, ^4\text{P}$ states of the ionised atom can be calculated⁶ to be greater than 2 eV. Our observations indicate a multiplet splitting of less than 0.8 eV, which implies considerable delocalisation of unpaired spin density away from this centre. We propose that this is achieved by the formation of a loosely-bound $\text{O}_2 \cdots \text{O}$ species, between an interstitial O and an O_2^- ion at an

adjacent normal lattice site. The formal similarity of this species to the well-known ozonide anion O_3^- , the existence of which in irradiated $NaClO_3$ has been proposed from ESR studies^{7,8} supports our assignment. It should be stressed, that the formation of the $O_2 \cdots O$ complex is strongly dependent on the volume of the interstitial cavity, which must only just be large enough to trap the O atom; thus we have failed to detect any O-trapping in the low temperature radiation-induced decomposition of $NaNO_3$ (R. G. C. and J. L. unpublished).

If we suppose a step-wise process for the decomposition¹ involving $ClO_3^- \rightarrow ClO_2^- + O$ as the initial step, then the majority of the oxygen atoms will diffuse through the lattice (low trapping probability for interstitial O) to form molecular oxygen, which either escapes from the lattice, or is trapped at anion sites as O_2^- . On continued irradiation interstitial oxygen atoms will also combine with O_2^- , resulting in a slow rise in the high binding energy O(1s) peak. At long irradiation times, however, the number of oxygen atoms produced will decrease, which, when combined with a structural change to the NaCl phase (with a smaller interstitial cavity), will lead to the observed decrease in intensity. The warming-up experiment, shown in Fig. 2, can thus be explained in terms of the decomposition of $O_2 \cdots O$, and thermal diffusion of oxygen atoms which combine to form molecular oxygen.

We also observed that irradiation at the lowest temperature attainable (~ 85 K) led to no O trapping; also samples irradiated at higher temperatures and containing significant amounts of trapped O behaved exactly as in the warming up experiments on cooling to 85 K, that is, disappearance of the high binding energy O(1s) peak accompanied by the evolution of O_2 . This suggests that a structural change occurs, to a more closely-packed lattice, in the region of 85 K.

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Carbon fibre structure by electrolytic etching

STUDIES of polyacrylonitrile (PAN)-based carbon fibres have demonstrated a concentric three-zone skin-sheath-core structure¹⁻⁷ originating in the oxidation of the precursor fibre before pyrolysis heat-treatment^{5,6}. I describe here an electrolytic oxidation treatment that is effective in showing up different zones in carbon fibre sections.

PAN fibres, 1.5 denier were oxidised in air at 230 °C for either 30 min or 2 h, and heat-treated at 1,000 °C, 1,400 °C or 2,500 °C giving in total six fibre types. Single tows of each fibre type were mounted in epoxy resin blocks and 3-mm thick disks were cut normal to the fibre axis. The disks were polished on one side and sputter-coated with gold on the other to make electrical connection to all fibre ends. For electrolysis the polished face was made anode in a 2 M sulphuric acid bath with carbon cathode. Longitudinal fibre sections were also prepared in a similar manner. In these, conduction through the disk is by fibre-to-fibre contact, and it was necessary to use a high-volume fraction composite of commercial type 2 PAN-based fibre rather than single tows in order to achieve sufficient conductivity. The electrolytic treatment is basically anodic oxidation. Average

current density at the fibre ends was limited to 140 A m⁻² for convenient treatment times, though neither acid concentration nor current density were found to be critical. Specimens were removed after intervals of 10–60 s, and rinsed in distilled water before microscopy.

In the initial stages of the treatment, fibre surfaces developed colours which later dulled as oxidative removal of carbon formed relief. The colours are believed to arise from interference in a thin surface layer with different optical properties, formed by intercalation of sulphuric acid into the fibre^{7,8}. As the optical path difference inferred from the interference colour increases linearly at a rate of 0.18 nm per C/m², the advancement of any particular area in the interference series is an indicator of its extent of reaction in forming an intercalation compound. The colours extend only to second order blue, which is maintained after relief is developed. Specimens were examined soon after etching as the colours fade over a period of several weeks at room temperature.

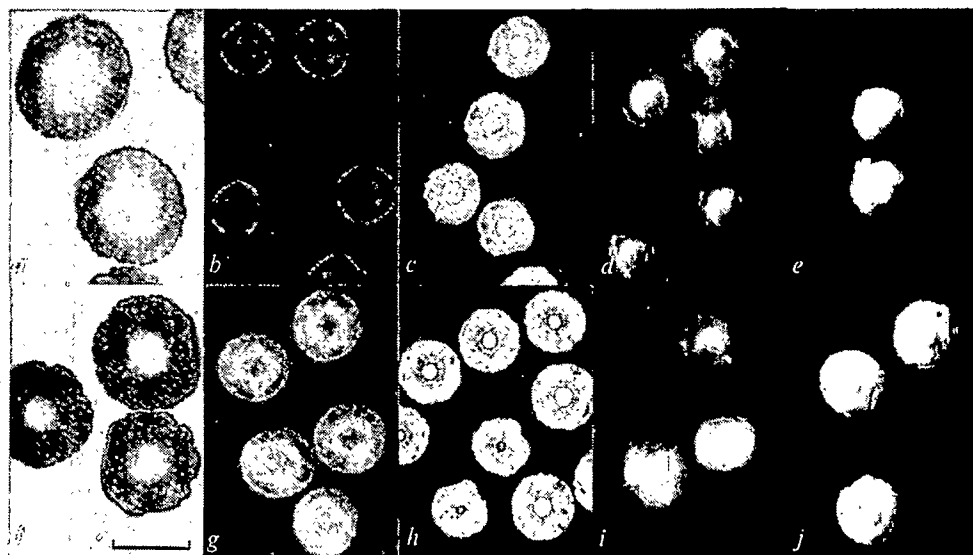
For all sections cut normal to the fibre axis the sheath etched more rapidly than the core, and increasingly so for higher heat-treatment temperatures (HTT) (Fig. 1*c-e, h-j*). In every case etching in the sheath decreases outwards, but there are differences in this gradation between HTT's. A dark ring of very etched material, approximately 1 μm wide, immediately surrounds the core in 2,500 °C HTT fibres, while the remainder of the sheath is uniformly etched at a lower rate. Fibres heat-treated at 1,000 °C and 1,400 °C show a more even outward decrease in etching of the sheath which is shown clearly by the (coloured) concentric rings in Fig. 2*b*, and the smooth contours in Fig. 1*d, e, i, j*. The core etched uniformly for all heat treatments and is seen as a plateau in fibres showing relief. In longitudinal sections both sheath and core etched uniformly, but the core more rapidly than the sheath, a reversal of the order in transverse sections (Fig. 2*a*).

Watt and Johnson⁵ have observed oxidation zones in thin sections of the PAN fibres from which the carbon fibres examined here were made. The thickness of the darker oxidised zone corresponding to the sheath increases with the square root of oxidation time (Fig. 1*a, f*) and after heat treatment the structure is apparent in polarised light^{2,3,6} (Fig. 1*b, g*). Direct comparison of the zone dimensions in oxidised and carbonised fibres is not possible because of the shrinkage from 13 to 8 μm diameter, but the relative sheath thicknesses correspond. In polarised light a Maltese cross is seen in both sheath and skin, whereas the core is optically isotropic. The contrast is lower in 1,000 °C and 1,400 °C HTT fibres but the pattern remains the same. These patterns have been interpreted in terms of the *c*-plane orientation about the fibre axis^{2,3}, which for the 8 μm PAN-based fibres is thought to be concentric in the skin, radial in the sheath and random in the core. This model agrees well with the structure seen on fracture surfaces² and in plasma-oxidised fibre sections¹, where radial striations may be seen in the sheath, and circumferential rings in the skin. Orientation is less apparent in these surfaces below 2,000 °C HTT, and it is noted that only in Fig. 1*h* (2,500 °C HTT) are there dark radial streaks in the sheath.

It is becoming evident that the sheath/core distinction is not simply one of *c*-plane orientation. Electron microscopy of crushed fibre fragments⁴, and electron diffraction studies of taper-thinned fibres² show decreasing layer-plane misorientation and decreasing crystallite stacking height from the fibre centre outwards. A uniform outward decrease is associated with the lower heat-treatment temperatures, but for 2,500 °C HTT fibre the transition takes place in a narrow 1-μm wide band; patterns which are mirrored in the etched fibre surfaces (Fig. 1*c-e, h-j*).

The thickness of the intercalated layer might be expected to vary with the inclination of the *c* planes to the surface, rising to a maximum when they are normal. If there are preferred circumferential and radial crystallite orientations any non-diametric longitudinal section will show some variation of the mean *c* plane to surface angle within zones, which might be reflected in variation of the etching rate across the zones. In fact, the zones etch uniformly (Fig. 2*a*), showing that the technique is insensitive to such orientation as may exist. Differences in crystallite mis-orientation in sheath and core will give rise to differences in plane

Fig. 1 Transverse fibre sections, air-oxidised at 230°C for 30 min (a-e) or 2 h (f-j). a,f, Thin sections of PAN fibre before heat-treatment. b,g, Polarised light, 2,500 °C HTT. c,h, Etch colours in 2,500 °C HTT fibres. d,i, Etch relief in 1,400 °C HTT fibres. e,j, Etch relief in 1,000 °C HTT fibres. Scale bar, 10 μ m.



edge density in transverse sections, but these do not seem able to account for the contrast in etch rates.

In longitudinal sections intercalation will initially take place in crystallites presenting *c*-plane edges to the surface, and the depth of penetration will be limited by the crystallite width. A higher etching rate will then be associated with wider crystallites. The core is known to have greater crystallite thickness than the sheath, and it is possible that the effective crystallite width is also greater, which would account for its higher rate of etching.

Because of the high axial *c*-plane alignment all crystallites in transverse sections will be suitably oriented for intercalation. But, the rate of intercalation is expected to be greater in the less misoriented sheath where there is greater separation of lattice distortions hindering penetration. It is probable also that axial curvature and folding of layer planes produces channels in which intercalation is facilitated, and a higher density of these may accompany the smaller sheath crystallite thickness.

These ideas come some way to explaining the reversal of sheath and core etch rates in longitudinal and transverse sections. Although the etch patterns can only be correlated with what is already known of fibre fine structure, the technique is worth pursuing as a simple and sensitive monitor of morphological variations arising during the processing of PAN fibre to carbon fibre. As such it is an aid in relating strength properties to structure, and for this application a particular advantage of

current controlled etching is the fact that insulating resin matrices are not attacked.

I thank W. Watt and W. Johnson of the Royal Aircraft Establishment, Farnborough for useful discussions and for providing carbon fibres and the photographs of PAN fibres.

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Received 17 October; accepted 8 November 1977.

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Fig. 2 Etched sections of commercial type 2 PAN-based carbon fibres. a, The two centre fibres show a dark core more etched than the sheath. b, Concentric (coloured) rings in the sheath indicate a decrease of etching outwards. Scale bar, 10 μ m.



Evidence for reptation in an entangled polymer melt

ENTANGLEMENTS, their nature and their role in the dynamic properties of concentrated polymer solutions and melts are not well understood^{1,2}. The classical molecular view of entanglements has been one of rope-like intermolecular couplings at a number of points along the length of a molecule; molecules in motion would drag past these couplings, the essential effect being one of enhanced friction^{1,3}. There has been a growing realisation that this model is inadequate^{2,4,5}. The essence of the problem, rather, seems to be that of the topological restrictions imposed on the motion of each polymer molecule by its neighbours: movement of a given polymer chain is constrained at the points of entanglement or intersection with adjacent chains². Theoretical treatment of the topological problem is difficult⁶, and has met only with limited success⁵. An interesting proposal regarding the motion of molecules within entangled polymer systems has been put forward by De Gennes^{4,7}: according to this, the motion of a given polymer molecule is confined within a virtual 'tube' defined by the locus of its intersections (or points of 'entanglement') with adjacent molecules (Fig. 1). The molecule is constrained to wriggle, snake-like, along its own length, by curvilinear propagation of length defects such as kinks or 'twists'⁸ along the tube; this mode of motion has been termed reptation⁴ (from reptile). Reptative motion clearly satisfies the central requirement of entangled systems: that of the non-crossability by a given chain of the contours of its adjacent

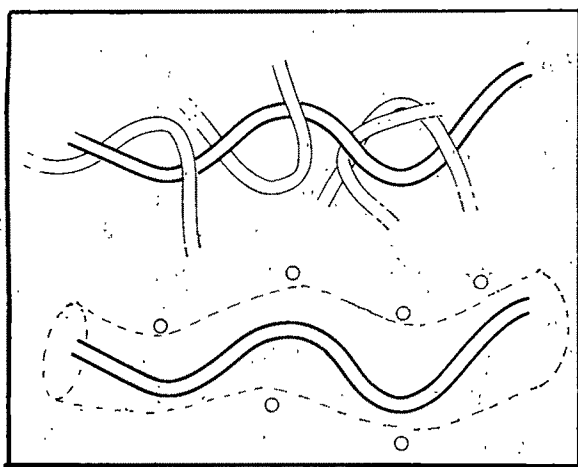


Fig. 1 A polymer chain in an entangled melt (or concentrated solution) may be regarded as being within a virtual 'tube' defined by the locus of its intersections with other melt molecules, as indicated. The obstacles defining the 'tube' in the lower figure are sections through the melt molecules in a plane parallel to the paper. For clarity, the density of segments shown is much lower than would be the case in a real melt.

neighbours. In a real polymer melt the topological environment of any given molecule (that is, the virtual 'tube' surrounding it) will itself change with time. This is because the adjacent molecules defining it are themselves mobile. If this reorganisation is sufficiently slow then the translational motion of the enclosed molecule will be effectively curvilinear (reptative). Consideration of the problem⁹ suggests that this will be the case in an entangled system. One then expects translational diffusion to be dominated by reptation. There is no direct experimental evidence supporting the physical reality of curvilinear motion in entangled polymer systems. I report here the results of experiments on diffusion within a polyethylene melt critically designed to test the reptation concept.

One outcome of constraining a polymer chain of random walk configuration to diffuse, by Brownian motion, within a confining (virtual) tube, is that the translational diffusion coefficient D scales as

$$D \propto M^{-2} \quad (1)$$

where M is the molecular weight. This may be readily proved⁴, and is a direct consequence of imposing a random walk configuration on the essentially one-dimensional curvilinear diffusion of the molecule along its own contour.

Previous studies of self-diffusion in concentrated polymer solutions and in melts have involved mainly the use of NMR¹⁰⁻¹⁴ and radio-tracer techniques¹⁵⁻¹⁷. The results of the NMR studies are ambiguous, as it is difficult to separate the contributions to the overall motion due to translation of the molecule as a whole from those due to the internal (segmental) motion^{11,13}. McCall *et al.*¹⁰, for example, using the NMR

technique, have estimated the diffusion coefficient in molten polyethylene to vary as $D \propto M^{-5/2}$; their result, however, was based on rather limited data. In the case of the radio-tracer studies involving the variation of D with diffusant length in polymer melts, the results^{15,16} have been of a somewhat qualitative nature and have lacked a discriminative character.

In the present experiments the translational diffusion coefficient D of a series of deuterated linear polyethylene (DPE) fractions was measured in a protonated linear polyethylene (PPE) melt. Neutron scattering studies^{18,19} of polyethylene melts, in which small amounts of DPE were dissolved in PPE (and vice versa), indicate that the radius of gyration of the minor component in the melt is consistent with its having a random coil configuration. Thus if translational diffusion of the DPE molecules within the PPE melt is taking place by reptation, the relation of equation (1) must hold: and this is what I set out to test.

The measuring technique was developed by B. J. Briscoe and myself, and is based on infrared (IR) microdensitometry; it is described in detail elsewhere²⁰. In essence, the technique involves setting up a concentration step-function of the diffusant of interest within the matrix, and allowing it to broaden, with time, under diffusion. Measurement of the final diffusion broadened profile is carried out using an IR microdensitometer, which yields a value for D . The diffusant must absorb at some IR frequency unaffected by the matrix: in the present case, the labelling frequency was that of the C-D stretching mode (in DPE) at $2,170 \text{ cm}^{-1}$, a region free of absorption by the PPE.

Five fractions of linear DPE (98% deuterated) were used. Table 1 shows their molecular weight characteristics, as determined by gel permeation chromatography. The PPE matrix was linear polyethylene of $M_w = 1.6 \times 10^5$, $M_w/M_n \approx 15$ and $\text{CH}_3/1,000\text{C} < 1$; this molecular weight is much higher than the critical molecular weight for the onset of 'entangled' behaviour, as deduced from viscosity and other measurements^{1,2}. Solid solutions, 2% w/w, of each DPE fraction in the PPE matrix were prepared by dissolving and mixing both components in boiling xylene; the mixture was then precipitated from solution by pouring into cold excess methanol. After washing and drying, pellets were moulded from these precipitates and from pure PPE which had been similarly dissolved and precipitated, and these were coherently joined²⁰ to form composites containing a step function in DPE concentration (Fig. 2a). The composites were placed in suitable PTFE containers inside sealed glass ampoules under nitrogen, and left for various lengths of time at the diffusion temperature. On quenching (thereby effectively stopping further diffusion), slices were taken from the composites and scanned in the IR microdensitometer²⁰ to yield the diffusion broadened profiles (Fig. 2b).

Evaluation of D for a diffusion broadened step function is straightforward when dealing with a single diffusing species²¹. When a diffusant is not monodispersed, however, difficulties arise in interpreting the experimental data. This problem has led to serious anomalies in previous studies of self-diffusion, mainly with NMR techniques^{11,12}. In the present case, the final broadened profile (such as in Fig. 2b) is the sum of contributions from species of different molecular weight (within each DPE fraction, see Table 1), and hence different D values. If a diffusing species of molecular weight = M_i has a diffusion coefficient D_i associated with it, such that $D_i \propto M_i^{-\alpha}$, then analysis shows (J.K. in preparation) that the slope of the broadened step function at the position of the original interface (Fig. 2b) is the experimental quantity which is to be measured. This yields an overall diffusion coefficient \bar{D} associated with a diffusion-average molecular weight, M_D , such that

$$\bar{D} \propto M_D^{-\alpha} \quad (2)$$

where

$$M_D = \left(\sum_i w_i M_i^{-\alpha/2} \right)^{-2/\alpha}$$

Characteristics were determined by gel permeation chromatography. N_w is the weight-average degree of polymerisation, corresponding to M_w .

Table 1 Molecular weight characteristics of the deuterated polyethylene (DPE) fractions.

Fraction	M_w	M_w/M_n	(N_w)
DPE1	3,600	2.25	(225)
DPE2	4,600	2.2	(288)
DPE3	11,000	3.4	(688)
DPE4	17,000	2.2	(1,063)
DPE5	23,000	1.8	(1,438)

w_i are the weight fractions of each species M_i comprising the diffusant sample.

In Fig. 3 M_w is plotted against the diffusion coefficient \bar{D} as measured from the slope of the diffusion-broadened profiles such as in Fig. 2b. The best fit to the data is

$$\bar{D} \propto M_w^p \quad (3)$$

where $p = -2.0 \pm 0.1$. But for $\alpha = -2$, M_D becomes identical with M_w : for consistency of equations (2) and (3), therefore, α must be identified with p .

Thus for a DPE molecule of molecular weight M , diffusing in an 'entangled' PPE melt, the translational diffusion coefficient D scales as

$$D \propto M^{-2 \pm 0.1}$$

This result is to be compared with the reptation relation of equation (1); it may be contrasted with the relation $D \propto M^{-3.5}$ expected on the basis of, for example, Bueche's entanglement-coupling model.

These results provide direct experimental support for the suggestion that the translational diffusion mode of polymer molecules in 'entangled' melts is a curvilinear one. This has a number of implications.

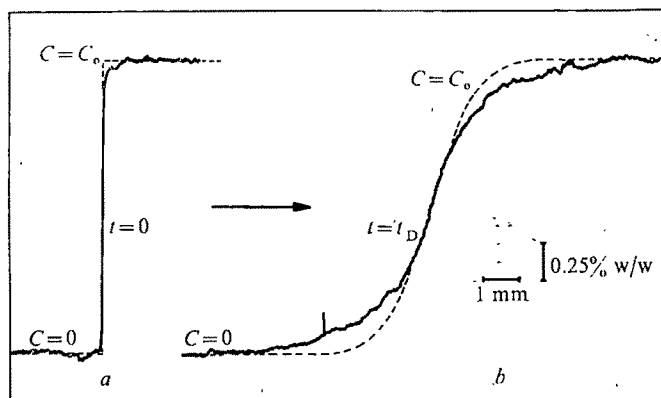


Fig. 2 Typical concentration profiles of deuterated polyethylene (DPE) in the protonated polyethylene matrix (PPE). *a*, Immediately subsequent to creation of a step-function. $C_0 = 2\%$ w/w of fraction DPE3 in PPE. The broken line represents an ideal step-function, and the continuous line is the profile as scanned in the infrared microdensitometer¹⁸. The scanning frequency is that of the C-D stretching mode at $2,170 \text{ cm}^{-1}$. *b*, The concentration profile of (*a*) following a diffusion run. Diffusion temperature $T_D = 176.0 \pm 0.3^\circ \text{C}$; $t_D = 1.114 \times 10^6 \text{ s}$; D as evaluated from the slope at the position of the original interface, is $3.7 \pm 0.6 \times 10^{-9} \text{ cm}^2 \text{ s}^{-1}$. The broken curve is the theoretical Fickian profile for a single diffusing species with the diffusion coefficient D ; the deviations from the experimental profile are due to the polydispersity of the actual diffusant sample (see text and also equation (2)).

The effect of 'entanglements' on the slow relaxation modes in 'entangled' systems now seems to be clearer: the slowest of these modes corresponds to the centre-of-mass translational diffusion of the molecules, and is constrained to take place by reptation. The concept of localised entanglement coupling at a relatively small number of points, which lay at the heart of classical theory, must give way to the view where the effect of entanglements is one which pervades the length of a molecule: the net result is one of topological constraints on the motion of molecules. For sufficiently high molecular weight, curvilinear motion alone is allowed.

It is tempting to suggest, in view of these results, that the onset of snake-like motion corresponds also to the onset of entangled behaviour. This suggestion is explored elsewhere⁹. Within the range of experimental parameters described,

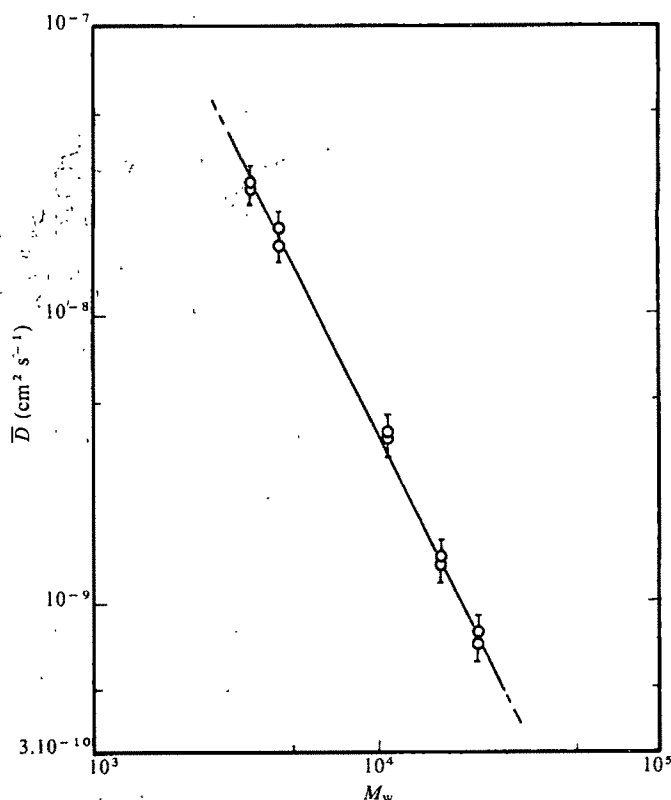


Fig. 3 The variation of \bar{D} with M_w for deuterated polyethylene diffusing in a protonated polyethylene matrix, at a diffusion temperature of $176.0 \pm 0.3^\circ \text{C}$. Each point represents a separate experiment, and is the mean of 10 separate profiles such as in Fig. 2b. The lengths of experimental runs (t_D) vary by a factor of ~ 3 within each pair of experiments for a given DPE fraction. The least-squares best-fit to the data is the relation:

$$\bar{D} = 0.2 M_w^{-2.0 \pm 0.1}, \text{ and is shown.}$$

however, the results clearly support De Gennes' notion of reptation in an entangled polymer system.

I thank Professor Sir S. F. Edwards for stimulating discussions, Dr D. G. Ballard and Dr G. Longman for supplying the deuterated fractions, and Professor D. Tabor and Dr B. J. Briscoe for their encouragement. A grant from Dow Corning Chemicals and a Research Fellowship from St Catharine's College, Cambridge, are both gratefully acknowledged.

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Received 7 July; accepted 4 November 1977.

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Improving the timbre and responsiveness of a bass trombone

THE design of brass instruments has always been a largely heuristic procedure. Recently, however, a knowledge of the acoustic impedance of the instrument has been used to correct faulty intonation in a trumpet¹. Here we demonstrate how such knowledge may be used to improve both the timbre and the responsiveness (or 'feel') of a bass trombone.

Bass trombones commonly use one or more valves (in addition to the slide) to increase the length of the instrument, thereby reducing the playing frequency. The instrument used in this experiment possesses a 'G valve' which lowers the pitch of a Bb instrument by three semitones. It follows, therefore, that the note G2 (98 Hz) may be played in two ways, either (1) the slide in 1st position and the valve depressed; or (2) the slide in 4th position and the valve released. In both cases the overall length of the instrument is the same, but the extra tubing is introduced at different points. J.M.B. noted that using the valve to play G2 resulted in a note that was difficult to sustain comfortably, and that this difficulty further manifested itself in a marked deterioration of the timbre.

The acoustic impedance of the instrument with all its tuning slides fully in was measured for both of the above cases, using apparatus described elsewhere². The results are shown in Fig. 1. The plots are similar, but the fundamental of G2 (which is the second maximum) is flatter by 6 Hz using the valve. There are also differences between the amplitudes of the maxima, but we shall confine ourselves to matching the frequencies of the fundamental as closely as possible for the two cases. The need to do so is explained by Benade³. Since the sound generating mechanism of brass instruments is non-linear, it may be shown that all the maxima which lie close to integral multiples of the playing frequency interact to sustain a note to an extent depending on the playing dynamic. This interaction is referred to by Benade as a "regime of oscillation". When using the valve to play G2 the fundamental is too flat for a truly successful 'regime' although the remaining maxima are reasonably well positioned. What is required then, is to raise the frequency of the fundamental when the valve is used without altering the positions of higher maxima.

Wogram⁴ introduces the concept of a 'sum function' which he calculates by determining the real part of the input impedance, and then summing the input resistance values at harmonics of a given fundamental frequency. He further shows that the peaks of this 'sum function' are simply related to the playing frequencies of an instrument,

Fig. 1 The acoustic impedance of the instrument before modification. Using the valve (dashed line); not using the valve (solid line).

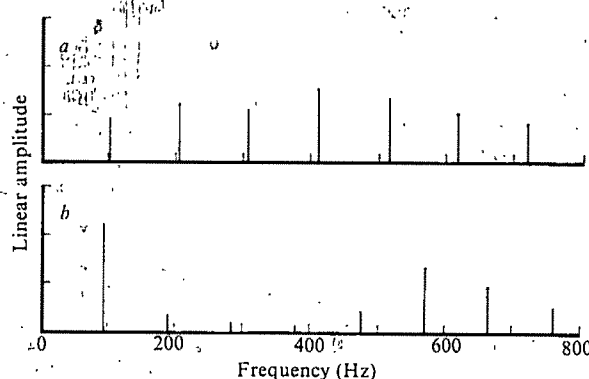
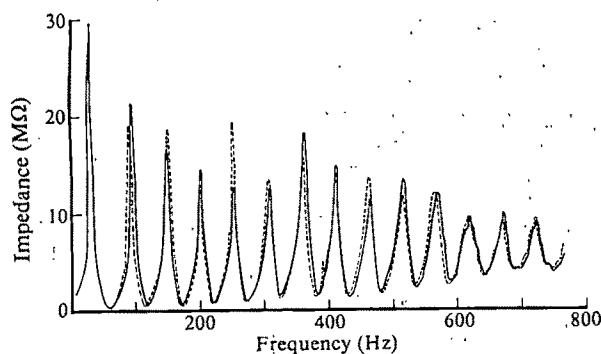


Fig. 2 The internal spectra of the modified instrument *a*, ff; *b*, pp.

and thus enable one to study the way the playing frequency changes with playing dynamic in a systematic manner. We have also written a computer program which calculates the internal spectrum for any playing frequency but in particular the ones predicted by the peaks of the 'sum function'. When the 'sum function' is calculated for the bass trombone using the valve it is found that at high dynamic levels the playing frequency is 103 Hz but at low levels it falls to 94 Hz and the internal spectrum envelope deteriorates considerably (see Fig. 2). Interestingly the change of playing frequency also appears when 4th position is used, though to a smaller extent, indicating that the possibility still remains of affecting further slight improvements to the whole instrument.

A perturbation theory exists to move the positions of the resonances of the trumpet¹ and a simple scaling procedure enables it to be used for a trombone. Clearly it is necessary to restrict the changes only to the G valve section so that the parts common to the Bb and G instruments are unaffected. Calculations indicated that a 9% reduction in the cross-sectional area of the tube forming the G valve was required. A new section was, therefore, fitted, and the acoustic impedance was again measured. The results indicate a considerable improvement although the fundamental is still 3 Hz too flat.

To determine whether any real subjective improvement had been made, a professional bass trombone player was invited to compare this instrument with an unmodified one whose impedance curve was measured and found to be identical (within experimental error²) to the results shown in Fig. 1. The player wore a blindfold and heavy gloves during the experiment, and the slides of both instruments were cleaned and oiled so that they felt as similar as possible. (This precaution is necessary because musicians are strongly influenced by the slide quality⁵.) The player was presented with a pair of instruments sequentially. Each pair was either a repetition of a given instrument or the two different instruments. The player was then instructed to play the note G2 under both conditions (with and without the valve) and then state whether he thought they were the same or different. If he thought they were different he was then asked which one of the pair he preferred. During extensive trials he could always distinguish between the two different instruments, and always correctly identified the repetitions. He referred to the unmodified instrument as "stuffy", "dull", and "hard to play" when using the valve. He greatly preferred the modified instrument and stated that the two playing conditions were "as closely matched as possible".

Thus the timbre and responsiveness, in addition to the intonation, may be improved given an apparatus for measuring acoustic impedance, and a theory which relates the geometry of the instrument to the frequency distribution of the impedance maxima.

This work is supported by grants from the SRC and Boosey & Hawkes Ltd.

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Guinea Current upwelling

THE historical record of maritime observations are summarised here to indicate the dominant seasonal variations in upwelling, and in certain associated processes, within the Guinea Current region. The region is similar to other eastern ocean boundary upwelling areas in the appearance of cool sea temperatures near the coast, productive coastal fisheries, and a zone of low rainfall on the adjacent coast (Fig. 1). It differs from some of the more studied regions in several important respects. These include the zonal rather than meridional trend of the coast, the influence of a rather narrow intense coastwise current, and an unusual lack of correspondence on the seasonal time scale between sea-temperature features attributable to upwelling, and features in the overlying wind stress field¹. There seems to be a link between interyear variations in upwelling intensity and corresponding variations in both coastal rainfall and local fishery success.

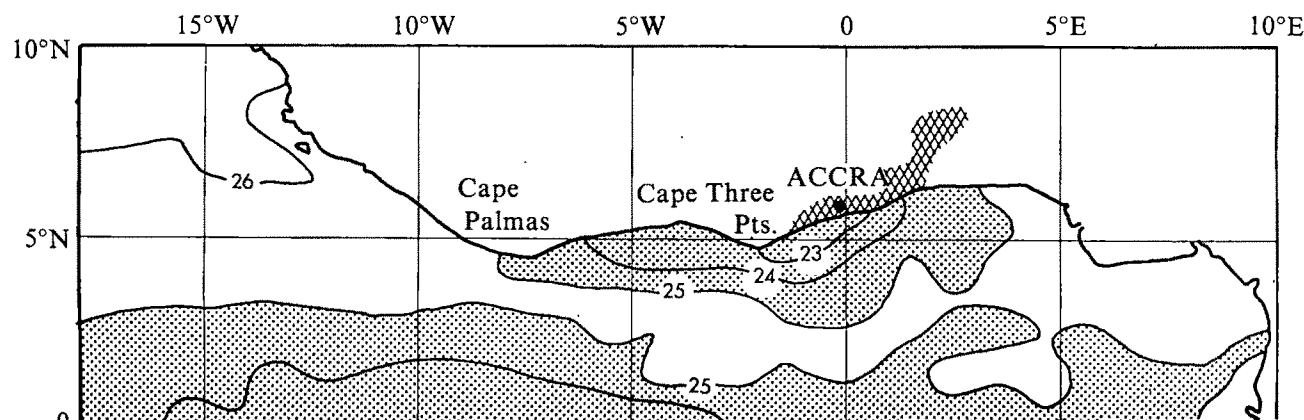
To construct the sea-temperature distribution shown in Fig. 1, maritime reports for the period 1850–1970 were obtained from the U.S. National Climatic Center's file of marine surface observations (TDF-11), checked for gross errors, and summarised by month and by 1° 'square' areas. Mean values were contoured by hand, taking into account the abundance of observations and magnitude of standard deviations. Data abundance was greatest near the coast and to the west of Cape Palmas. The effect of warm advection in the eastward-flowing Guinea Current is apparent in the shape of the 26 °C isotherm and in the offshore temperature

maximum which separates the cool temperatures along the coast east of Cape Palmas, attributable to coastal upwelling, from cool temperatures near the equator which are probably related to equatorial upwelling.

The seasonal distribution of sea-surface temperature within a line of 1° square areas stretching southward from the coast near Cape Three Points (Fig. 2) features an offshore maximum every month, suggesting continuation of some degree of coastal upwelling throughout the year. The offshore gradients are most intense from June through October; coolest coastal temperatures are reached during August and September. A secondary coastal minimum appears during January, resulting in a two-peaked annual temperature cycle.

A similar display of the seasonal cycle of sea-surface temperature (Fig. 3), this time within a line of 1° square areas arranged along the coastal boundary, reveals continuity of the summer minimum along the entire north coast of the Gulf of Guinea. In Fig. 3, west is towards the top (see Fig. 3d); thus the direction of flow of the Guinea Current is from the top towards the bottom of the diagram. The temperature decreases in the direction of flow to an absolute minimum east of Cape Three Points. Although the contour interval in Fig. 3 is too coarse to show it, much of the temperature decrease occurs in two abrupt steps upon passing each of the two major capes, Cape Palmas and Cape Three Points. Particularly sharp temperature gradients have been reported off Cape Palmas². The coolest region, defined by the 24 °C isotherm, appears in Fig. 3 to be split into two lobes by slightly higher temperatures from 0–1°W longitude; this is an artefact due to that particular 1° square being displaced slightly offshore relative to the squares to either side. Eastward of this late summer minimum feature, sea-surface temperature increases towards a region increasingly affected by outflow from the Niger River Delta and other sources. A cold temperature feature is apparent during winter in the extreme western portion of the region. The sharp gradient near 15°W longitude indicates the southern limit of the tropical front which oscillates seasonally along the west African coast between 10°N and 20°N latitudes, apparently responding to seasonal variations in wind-induced upwelling and associated along shore advection³. Extending eastwards from the front is a winter temperature minimum which retains its identity along the Guinea Coast well into the Bight of Biafra (~7°E longitude) where it is evident as a slight reversal during January. The two seasonal temperature minima are separated by periods of relatively warm sea-surface temperatures, with the major maximum occurring during spring.

Fig. 1 Long-term mean distribution of sea-surface temperature, summarised by 1° square areas for August. The contour interval is 1 °C; temperatures less than 25 °C are shaded. The crosshatched area on the coast in the vicinity of Accra indicates an area of less than 40 inches annual rainfall, and defines the approximate extent of the Accra dry belt¹⁶.



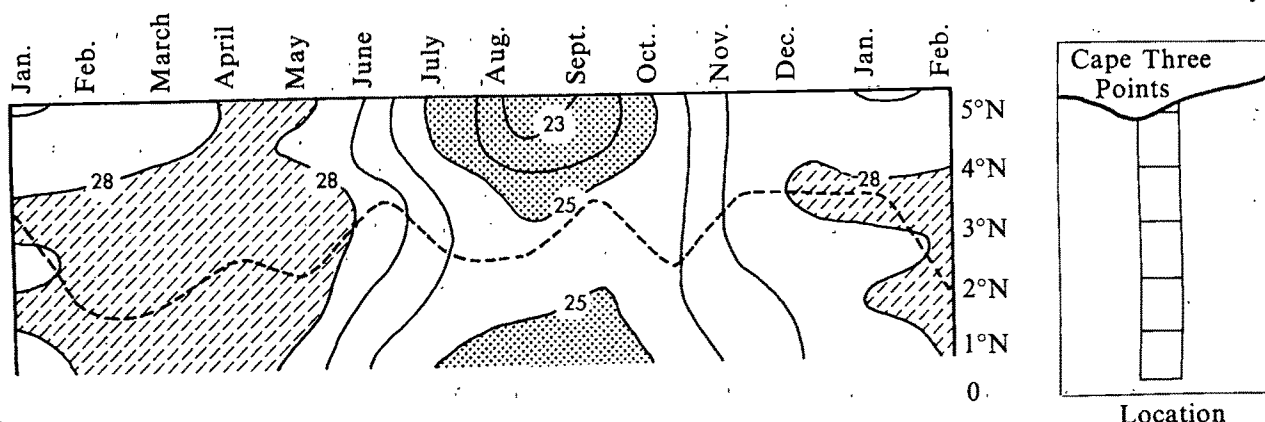


Fig. 2 Long-term composite annual cycle of sea-surface temperature within the line of 1° squares normal to the coast near Cape Three Points shown to the right. The contour interval is 1°C ; temperatures less than 25°C are shaded, and those greater than 28°C are indicated by broken diagonal hatching. The location of the maximum mean temperature within the line of squares for each long-term monthly sample is indicated by the dashed line.

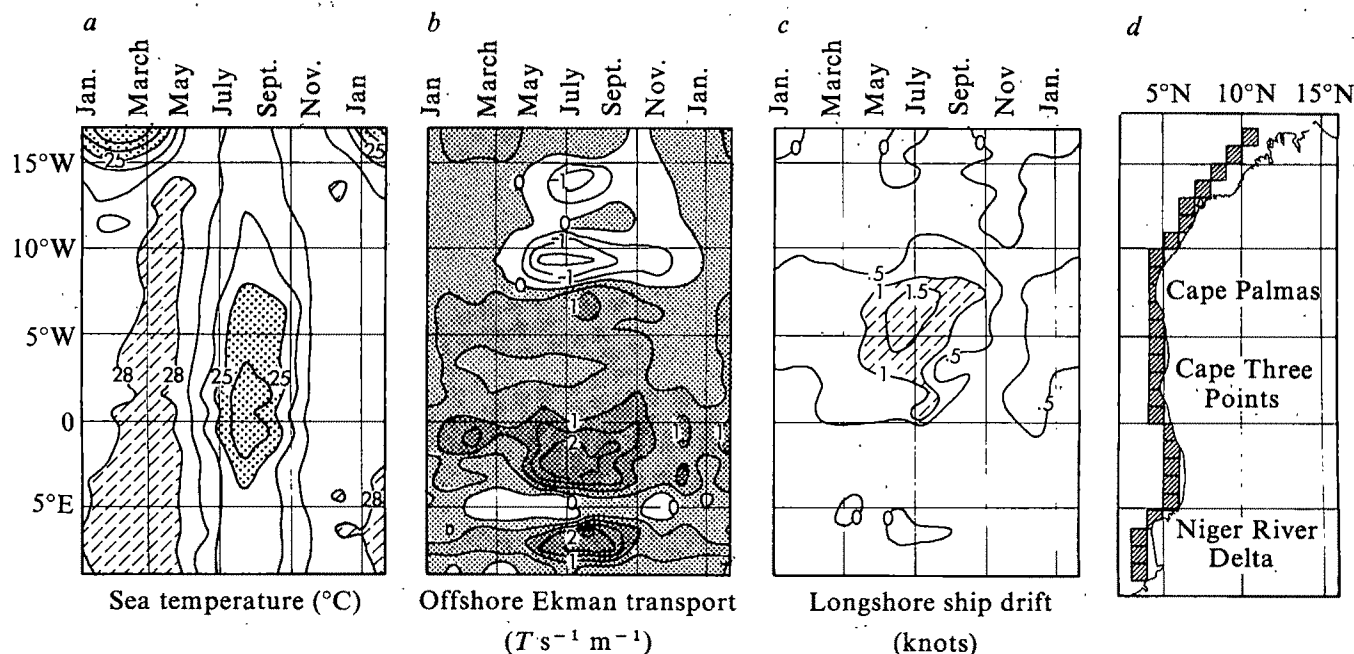
Being near the equator, the Guinea Current region experiences two periods of vertical sun per year, one shortly before the vernal equinox and the other shortly after the autumnal equinox. Unlike the situation in more temperate latitudes, both solstices are periods of low sun. Thus, the double peaked seasonal cycle of sea temperature matches qualitatively the cycle of solar height. But compilations of mid-ocean sea-surface temperatures¹, at similar latitudes where the cycle of solar height is identical, indicate no such strong correspondence. Thus, it is difficult to ascribe the observed features directly to such large scale effects as passages of the Sun. Rather, they would seem to be almost entirely controlled by processes local to the Guinea Current region.

In the classical model for coastal upwelling², water set in motion by the stress of the wind is deflected offshore by the rotation of the Earth. When this occurs along an extensive stretch of coast such that the water transported off

the coast cannot readily be replaced by convergence of horizontal alongshore flow, replacement can occur by upwelling of deeper water.

Previous comparisons of offshore Ekman transport distributions with corresponding temperature distributions in the California Current³, and Canary Current⁴ regions on a similar 1° scale have yielded general conformities in major features, indicating seasonal variation in the local wind as the principal factor controlling seasonality in the coastal upwelling process. Accordingly, wind reports from the Guinea Current region were assembled from the same source as the temperature reports. These were converted to measures of sea-surface stress by squaring each reported wind speed and multiplying by the density of air (assumed constant, 0.00122 g cm^{-3}) and a constant drag coefficient (0.0013). The derived stress 'reports' were then averaged by components to yield a resultant mean stress for each long term composite month and 1° square area. This was con-

Fig. 3 Long-term composite monthly variations within the 1° square areas arranged along the coast, shown in (d). a, Sea-surface temperature. The contour interval is 1°C ; temperatures less than 25°C are shaded and those greater than 28°C are indicated by broken diagonal hatching. b, Offshore-directed component of Ekman transport. The contour interval is 0.5 tonnes S^{-1} transported across each 1 m width. Positive values, indicating seaward transport, are shaded; darker shading indicates more intense seaward transport. c, Alongshore component of ship drift. The positive direction is such that the coast is on the left when facing downstream. The contour interval is 0.5 knots ; speeds greater than one knot are indicated by broken diagonal hatching.



verted to Ekman transport, directed 90° to the right of the stress vector, by dividing by the Coriolis parameter computed at the mid latitude of the 1° square. Where the centre of the square is less than 5° from the equator the value of the Coriolis parameter at 5°N latitude was used. Offshore components were resolved along characteristic offshore normal directions, based on the nearshore bathymetry within each individual square.

The seasonal pattern of offshore-directed Ekman transport (Fig. 3) reveals certain features which correspond to features in the temperature pattern. Transport tends to be offshore throughout the year, except during certain months off Liberia and Sierra Leone ($8\text{--}14^\circ\text{W}$) and near the west-facing portion of the Niger River Delta shoreline ($4\text{--}6^\circ\text{E}$). A winter maximum of offshore transport occurs in March at the northwestern end of the area (top of the diagram), and retains continuity toward the east, shifting to February off Cape Palmas and Cape Three Points. This winter maximum generally corresponds to the winter temperature minimum described above. During the summer there is a relative maximum of offshore Ekman transport on the east side of Cape Palmas and another increase to the east of Cape Three Points; these correspond to the major decreases in sea temperature near these capes.

Maximum values of offshore transport occur to the east, however, that is downstream in relation to Guinea Current

observations produced as part of this study show a very significant component of the mean wind to be directed onshore at all seasons. Any larger portion of the transport being directed with the wind would, therefore, only imply a further reduction in local wind forcing of upwelling at the coast.

Figure 3c summarises reports of drift attributed to ocean currents compiled from ship's logbooks by the U.S. Naval Oceanographic Office. Although the number of available reports is very small, being of the order of 20 per month-square in the area from Cape Palmas to Cape Three Points, a coherent picture of the seasonal cycle of flow in the Guinea Current is apparent. Off Cape Palmas the current near the coast intensifies dramatically, diminishes slightly toward Cape Three Points, and drops off rapidly past Cape Three Points. Mean velocities near Cape Palmas vary from nearly 2 knots in July to less than half a knot in November. The current maximum is thus somewhat upstream of and slightly preceding the summer temperature minimum, actually coinciding in time to the period of most rapid temporal decrease of temperature and in space to the zone of most rapid spatial decrease. Horizontal advection in the current, of course, opposes the temperature decrease. Various mechanisms leading to vertical advection in an intensified current⁷⁻¹⁰ could perhaps contribute to the observed cooling. But, the lag of at least a month between

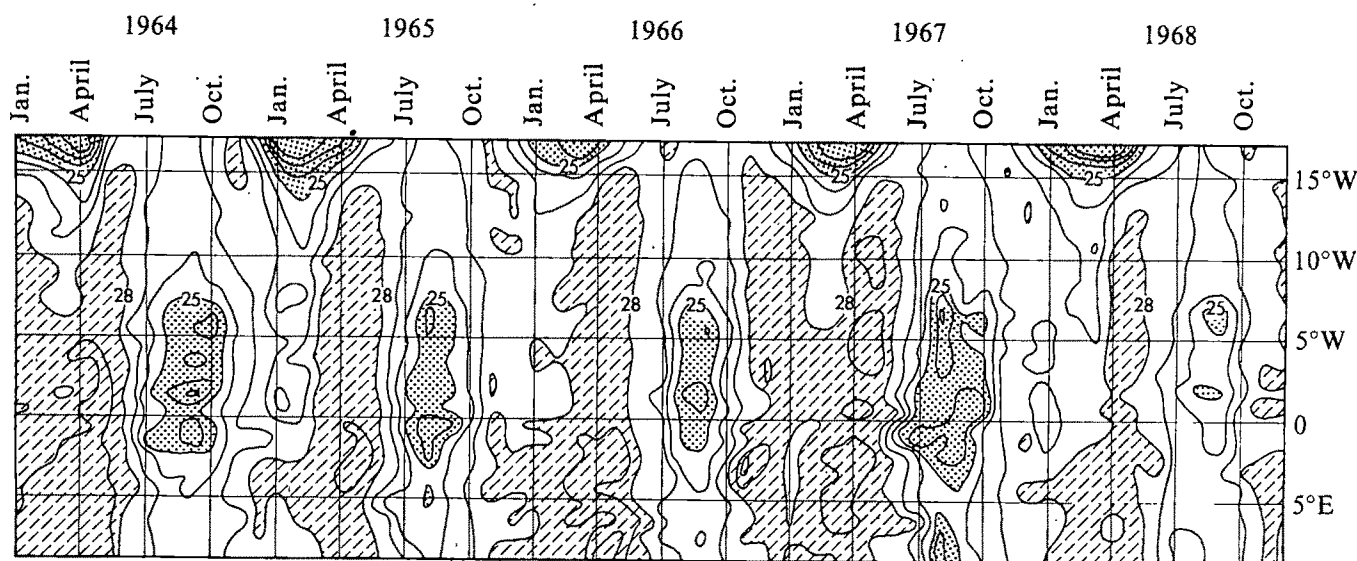


Fig. 4 Monthly temperature variation within the square areas shown in Fig. 3, during the years 1964 through 1968. The contour interval is 1°C ; temperatures less than 25°C are shaded and those greater than 28°C are indicated by broken diagonal hatching.

flow, of the location of summer minimum temperatures. The offshore transport maximum off Dahomey ($2\text{--}4^\circ\text{E}$) is in the area of most rapid down-stream warming. Likewise, a second offshore transport maximum in the Bight of Biafra ($6\text{--}9^\circ$) is not strongly reflected in the sea-temperature distribution, although both cases may involve some masking of the effects of upwelling by low salinity surface water. Certainly, there is nothing in the local Ekman transport distribution to account for the continuity of the temperature minimum to the west, that is, upstream, of Cape Palmas.

Since this region is very near the equator, the diminished Coriolis term in the dynamical equations may lead to a different balance of forces from that assumed in the Ekman transport relationship. In such a case the transport could be at a lesser angle than 90° to the direction of wind stress. Such an effect, however, cannot explain the non-correspondence of the distributions in Fig. 3. Summaries of wind

the maximum current speed in July and the minimum sea-surface temperatures in August–September (Fig. 3) is difficult to reconcile with any predominant effect of such processes; for example, at the indicated average current velocities, water upwelled near Cape Palmas during the July current maximum would be carried past Cape Three Points in less than 10 days.

Recent theoretical developments have indicated the ability of upwelling-produced displacements of the internal density structure to propagate in the form of coastal trapped waves, and thus to affect the temperature and current velocity fields at other locations along the coast^{11,12}. In the configuration of the Guinea Current region, the direction of propagation would be to the west. The degree of westward displacement of features in the sea temperature and drift patterns, compared to features in the Ekman transport field (Fig. 3), is consistent with computations from theory

Table 1 Comparison of intensity of summer-fall upwelling with July–October rainfall at three reporting stations in Ghana and with catch of the Ghanaian herring fishery for the years 1964 through 1968

		1964	1965	1966	1967	1968
Upwelling		High (late)	Intermediate	Intermediate	High	Low
Month		J A S O*	J A S O	J A S O	J A S O	J A S O
Rainfall (quintiles)	Accra	3 1 0 0	5 5 3 2	4 3 1 3	1 2 5 1	6 6 6 5
	Takoradi	2 4 1 0	4 4 5 1	5 4 2 2	2 2 3 1	6 6 6 5
	Kumasi	3 4 1 2	4 5 3 5	6 5 1 2	2 1 2 1	6 6 6 1
Summation		21	46	38	23	65
Herring catch (Ghana)		34.2	7.8	13.3	43.2	12.2

Upwelling intensity is estimated from sea-temperature distributions (Fig. 4). Rainfall is given in quintiles of the frequency group within which the recorded precipitation falls relative to a group of reference years¹⁷. 0 indicates monthly precipitation to have been lower, and a '6' indicates it to have been higher, than in any corresponding month in the reference series. In the line labelled summation the quintile values for the four months and the three stations are added together to give a seasonal index. The herring catch is in 10³ tonnes.

*J, July; A, August; S, September; O, October.

(A. J. Clarke, personal communication). Thus it seems that such processes may be exerting particularly strong control on the pattern of seasonal upwelling in the Guinea Current.

In the surface marine observation file (TDF-11) there is an abrupt increase in the density of reports in the Guinea Current region beginning with 1964. In the particular version of the file that was used in this study, however, the frequency begins to drop off rapidly after 1968. For these five years of maximum data density a time series of sea-surface temperature (Fig. 4) was constructed on the same basis as the long-term composite annual cycle (Fig. 3). Because of the higher short-term variance of the wind, the data density was not sufficient to derive a meaningful similar series of wind or Ekman transport variations.

If one examines the summer upwelling feature (10°W–5°E longitude) in Fig. 4, it is possible to class the summers of 1964 and 1967 as having been cold (strong upwelling), 1968 as having been warm (weak upwelling), and 1965 and 1966 as having been intermediate (Table 1). In addition the strong upwelling in 1964 seems to have occurred relatively late, the coolest monthly mean temperatures appearing in September rather than in August as is the case in long-term composite cycle (Fig. 3). In partial corroboration of this classification, dissolved oxygen data collected off Ivory Coast (~4°W) during the period 1966–70¹³ show low oxygen values characteristic of deeper layers unmistakably nearer the surface during the summer upwelling season of 1967 than in the other years of that particular series.

With these upwelling estimates, it is possible to look for apparent effects of upwelling variations on coastal rainfall. One mechanism which may contribute to the intense coastal aridity, which is a general characteristic of upwelling regions, is the stabilisation of the shoreward airflow by the cool upwelled surface waters, thereby inhibiting vertical development of storm clouds¹⁴. The Accra dry belt (Fig. 1), which has the appearance of a coastal enclave of semi-arid savanna surrounded by tropical rainforest, has been termed 'the most remarkable climatic anomaly of the Guinea coastlands'¹⁵. One of the unusual features cited is an anomalous dearth of rainfall during the late summer. Prevailing winds are from the south-west¹⁶; Figure 1 shows the Accra dry belt to be oriented directly downwind of the coldest sea-surface temperatures.

Table 1 classifies the rainfall recorded at three locations in Ghana¹⁷. Takoradi is located on the coast, just east of Cape Three Points. Kumasi is about 100 miles inland, between Takoradi and Accra. The correlation of the rainfall record with the upwelling estimates is convincingly strong. During the years of strong upwelling, 1964 and 1967,

the July–October rainfall was anomalously low; in fact 'late' upwelling estimated for 1964 corresponds to 'late' rainfall deficits in September and October. During the weak upwelling year, 1968, monthly mean rainfall actually exceeded the highest class intervals established from historical data at all three locations during the months of July, August, and September.

Also listed in Table 1 are the landings of herring (*Sardinella aurita*) in Ghana during these same five years¹⁸. This was Ghana's most important fishery during this period, largely operating from canoes very near shore during the summer-fall upwelling period¹⁹. Evidently periods of extremely good fishing were associated with strong upwelling, as are periods of subnormal coastal rainfall. During these years a sharply increased trend in fishing effort occurred, due to technology and so on. If this is taken into account in interpreting the landing figures, a relationship is even more apparent.

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Received 8 July; accepted 4 November 1977.

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The origin and relative abundances of C, N and the noble gases on the terrestrial planets and in meteorites

RASOOL and Le Sergeant¹ adopted the inhomogeneous accretion model for planets discussed by Turekian and Clark² and considered the question of whether carbonaceous chondrites or LL chondrites could better explain the relative abundances of volatile elements found on Earth and Mars. In the inhomogeneous accretion model the volatile elements of the terrestrial

planets are attributed to a component which is added late in the accretional history of the planet. Turekian and Clark² presumed this late-stage component to be like carbonaceous chondrites. But, Rasool and Le Sergeant¹ compared the relative concentrations of H, C, N, Ne, Ar, Kr, and Xe (normalised to C) for the Earth, C1 (carbonaceous) chondrites, and LL (ordinary) chondrites and concluded that relative volatile abundances for the Earth have greater similarity to LL chondrites than to C1 chondrites. Specifically, these authors noted that relative to carbon, abundances of the noble gases (Ne, Ar, Kr, and Xe) are within a factor of 3.8 for the Earth and LL chondrites, but differ by factors of 15–37 between the Earth and C1 chondrites. Abundances of H and N relative to C were indicated to be the same within a factor of 2.5 for both meteorite types and the Earth. Turekian and Clark² had previously made similar comparisons between the Earth and C1 chondrites and concluded that terrestrial volatiles were acquired from material having H/N/C ratios similar to C1 chondrites, but with order-of-magnitude higher abundances of the noble gases. Rasool and Le Sergeant, on the other hand, concluded that volatiles on Earth and, by extension of their arguments, also volatiles on Mars can better be explained by late accretion of material similar to LL chondrites rather than material similar to C1 chondrites. We disagree with the conclusion reached by Rasool and Le Sergeant and with the LL-chondrite data which they used to reach their conclusion. Our purpose here is to demonstrate (1) that the noble gas/C and noble gas/N ratios presently determined for the Earth differ by an order of magnitude from these ratios in most meteorites; (2) that as a group LL chondrites show no better agreement with the Earth in these ratios than do C1 chondrites; (3) data are too incomplete to lead to any conclusions about the type of meteoritic material which may have supplied volatiles to Mars. In addition we suggest that the noble gas/C and noble gas/N ratios for Venus are likely to be more like the Earth's than like meteorites.

Figure 1 plots ^{36}Ar concentrations against the $^{36}\text{Ar}/\text{C}$ ratio given in units of 10^{-8} cm^3 per g weight % for several types of meteorites. The major trend is for the $^{36}\text{Ar}/\text{C}$ ratio to decrease as the ^{36}Ar concentration decreases, which probably reflects either different formation temperatures, or the greater ease by which ^{36}Ar can be lost from material compared to C. The LL chondrites of petrographic grades 4–6 have ^{36}Ar concentrations two orders of magnitude lower and $^{36}\text{Ar}/\text{C}$ ratios a factor of ~ 1 –3 lower than carbonaceous chondrites. Other ordinary H and L chondrites of the same petrographic grades have very similar ^{36}Ar and C contents to the LL chondrites shown. Unequilibrated chondrites (ordinary chondrites of petrographic grade 3) have intermediate ^{36}Ar concentrations and seem to show the greatest range in $^{36}\text{Ar}/\text{C}$ ratios among the various meteorite types. But, the two highest $^{36}\text{Ar}/\text{C}$ ratios shown for unequilibrated chondrites (Krymka at 185 and Chainpur at 124) must be considered dubious, as separate ^{36}Ar analyses on these meteorites showed variations up to a factor of 10. A separate analysis of Chainpur gives the data plotted at $^{36}\text{Ar}/\text{C} = 20$. Ureilites, whose ^{36}Ar is associated with diamond and graphite, have the highest ^{36}Ar concentrations of any meteorite type. The two Murchison (C2) data points are acid-etched, nonsilicate residues containing $\sim 50\%$ organic polymer C, but comprising only ~ 1 –2% of the total meteorite. Several analogous acid-etched residues from Allende (C3) showed amorphous C contents of ~ 18 –97% and $^{36}\text{Ar}/\text{C}$ ratios of ~ 2 –10, with one value of 97 (ref. 4). No substantial data exists which indicates $^{36}\text{Ar}/\text{C}$ ratios in meteorites higher than $\sim 100 \times 10^{-8} \text{ cm}^3$ per g weight %.

In comparison to the meteorite data in Fig. 1, the ^{36}Ar concentration and $^{36}\text{Ar}/\text{C}$ ratio for the whole Earth have been estimated as $2.1 \times 10^{-8} \text{ cm}^3 \text{ g}^{-1}$ and 473 (10^{-8} cm^3 per g weight %) respectively², and plot far off Fig. 1 to the right. The $^{36}\text{Ar}/\text{C}$ ratios for the Sun⁴ and the atmosphere of Mars⁵ are larger than this ratio for the Earth by factors of 5×10^5 and 23, respectively. Individual meteorites have $^{36}\text{Ar}/\text{C}$ ratios lower by factors of 5–100 relative to the Earth. Considering the average $^{36}\text{Ar}/\text{C}$ of different classes of meteorites compared to the Earth, C1s are

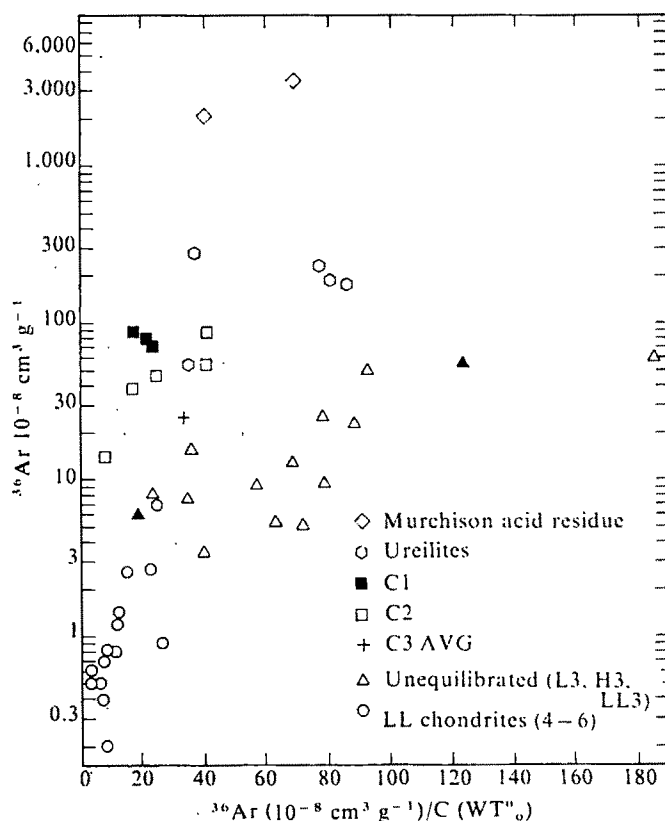


Fig. 1 Relationship between ^{36}Ar concentrations and $^{36}\text{Ar}/\text{C}$ ratios for several meteorite types. Units for $^{36}\text{Ar}/\text{C}$ are 10^{-8} cm^3 per g weight %, and are the typical analytical units reported for these elements. Each data point represents a separate meteorite except the two acid residue points for Murchison²⁷, two points for Chainpur LL3 \blacktriangle , and the averaged value for C3 chondrites. On this plot the Earth has a $^{36}\text{Ar}/\text{C}$ ratio of 473 and a ^{36}Ar concentration of 2.1 (ref. 2) and would plot far off the figure to the right. Meteorite data are taken from refs 7, 9, 10, 22, 25, 26.

lower by a factor of ~ 20 (as previously discussed²), C2s and C3s by a factor of ~ 14 , ureilites and unequilibrated chondrites by a factor of ~ 10 , and ordinary (LL) chondrites by a factor of ~ 45 . Ratios of Xe/C and Kr/C in meteorites are also lower than those ratios for the Earth by an order of magnitude or more. This conclusion arises from the fact that proportions of planetary Ar/Kr/Xe in various meteorite types are relatively constant, generally within a factor of 5 for Ar/Xe and a factor of 3 for Kr/Xe (refs 6–8), over a concentration range of three orders of magnitude. If anything ratios of Ar/Xe and Ar/Kr increase slightly with increasing ^{36}Ar concentration.

Neon in meteorites is more difficult to compare as it does not usually correlate with Ar. Neon in ordinary chondrites is almost entirely produced by cosmic ray interactions, and measurable amounts of planetary Ne has rarely been reported in these meteorites. Many individual meteorites representing essentially all meteorite types contain large quantities of neon implanted by the solar wind, and therefore are ill-suited for a comparison of planetary volatiles. Neon in ureilites is mainly produced by cosmic ray interactions, and the $^{20}\text{Ne}/^{36}\text{Ar}$ planetary ratio in ureilites is probably ≤ 0.01 (refs 7, 9). The unequilibrated chondrites also seem to contain little planetary Ne¹⁰. Even in carbonaceous chondrites the $^{20}\text{Ne}/^{36}\text{Ar}$ ratio varies by two orders of magnitude (~ 0.2 to 20) and indicates the presence of appreciable amounts of solar Ne in many samples⁷. The best estimate of the planetary $^{20}\text{Ne}/^{36}\text{Ar}$ mole ratio in carbonaceous chondrites is 0.28 (ref. 7), compared with the Earth value of 0.53 (ref. 2). With these values the $^{20}\text{Ne}/\text{C}$ ratio would be ~ 6 in carbonaceous chondrites and ~ 250 for the Earth. This is a difference of ~ 40 , which is essentially the value adopted by Turekian and Clark².

Available data, therefore, do not support the conclusion by Rasool and Le Sergeant¹ that the ratios of noble gases to carbon on Earth are much more similar to those of LL chondrites. The ³⁶Ar and C concentrations of LL chondrites, including the unequilibrated L3 chondrites, vary by factors of ~500 and ~22, respectively^{12, 13}. Thus the use of average or median concentrations from different populations instead of determinations for specific meteorites can be misleading. These authors reference the source of their noble gas and C data as two review papers. We surmise from their calculated ratios and from references given that they adopted a median C concentration of LL-chondrites¹² and an average noble gas abundance, which is also similar to a specific determination listed for Chainpur¹³. Chainpur is an LL chondrite which happens to be unequilibrated, and consequently has higher ³⁶Ar concentration and ³⁶Ar/C ratio than typical LL chondrites (Fig. 1). Various measured ³⁶Ar values for Chainpur also differ from one another and are lower by a factor of 4–20 than the LL chondrite ratio used by Rasool and Le Sergeant¹. The average ³⁶Ar/C of LL chondrites of petrographic grades 4–6 (Fig. 1) would be a factor of 45 lower than the ratio adopted by these authors. Similarly, the ²⁰Ne/C ratio adopted by Rasool and Le Sergeant for LL chondrites is much too high. As pointed out above, LL chondrites and unequilibrated chondrites (including Chainpur whose Ne content was apparently used by Rasool and Le Sergeant) rarely contain measurable amounts of planetary Ne (see, for example, refs 6, 10).

abundance pattern of volatiles on Earth could be derived from Ar-rich meteorites such as C1 chondrites by simple fractionation, as this would require greater loss of C relative to Ar, Kr, and Xe, and could be expected to fractionate Ar/Kr/Xe. The possibility of deriving the terrestrial volatiles from a component from which C had been selectively scavenged seems inconsistent with the relative nearness of the Earth to the Sun.

Rasool and Le Sergeant¹ also compared the relative abundances of volatile elements on Mars with their calculations for LL chondrites. Instead of normalising elemental ratios to C, for which the nonatmosphere inventory is unknown, they normalised to ³⁶Ar. They noted that the Kr/Ar and Xe/Ar ratios differed by only a factor of 3 between Mars volatiles and their LL chondrite values, and they used this similarity as evidence that volatiles on Mars were also introduced by a carrier similar to LL chondrites. But, such an argument based on the observed similarity in Kr/Ar and Xe/Ar ratios is trivial, because, as we summarised earlier, the Ar/Kr/Xe ratio is nearly constant for planetary gases in ordinary chondrites, unequilibrated chondrites, carbonaceous chondrites, ureilites, and the Earth. Thus, nearly constant Ar/Kr/Xe ratios would result no matter which of the above materials carried volatiles to Mars.

Another comparison is to consider the ³⁶Ar/N/C atom ratios in the atmospheres of Mars and Venus with these values for Earth and the meteorites (Table 1). The C and N abundances for meteorites and the Earth are measured and estimated values,

Table 1 Atom ratios of volatile elements in terrestrial planets and meteorites

Object	Ref.	N/C × 10 ⁻²	³⁶ Ar/N × 10 ⁻⁶	³⁶ Ar/C × 10 ⁻⁶	K/C	³⁶ Ar/K × 10 ⁻⁶
Earth*	2,21	4.6	5.5	0.25	1.8	6.3
Mars†	5	5.3	110	5.7	?	?
Venus‡	18	3.6	?	(200)‡	?	?
C1 chondrites§	7,22–24	3.7	0.33	0.012	0.0044	280
Ordinary chondrites	23–26	0.9–5	0.1–0.7	0.005	0.25	2.2

*K content of the Earth (minus the core) is taken as one-half chondritic²¹.

†Data for Mars and Venus are for atmospheres only. Reported data for Mars⁵ are assumed to be in volume %.

‡Total Ar/C ratio, where total Ar = ⁴⁰Ar + ³⁶Ar.

§N values used for C1s are recent measurements²², and are approximately a factor of 2 lower than previous values⁴.

||N abundances in ordinary chondrites show large variations^{22, 23, 25, 26}, and consequently only a range in N/C and ³⁶Ar/N is given.

What possible explanations exist for the observation that the Earth has considerably higher noble gas/C and noble gas/N ratios than known meteorite types (Fig. 1)? (1) Such ratios estimated for the whole Earth² may be incorrect. It is very unlikely that the abundances of Ar, Kr, and Xe for the Earth have been overestimated since the atmosphere contains the bulk of these gases, and the atmospheric abundances are known to a relatively high accuracy. The converse explanation, that the estimated abundances of C and N in the whole Earth² may be too low by a factor of 5–50, is perhaps possible. Essentially all of the estimated C in the Earth² is present as C in sediments (33 %) and ultramafic (mantle) rocks (~66%). Approximately 28 % of the estimated N in the Earth, on the other hand, is present in the atmosphere², and is known to a relatively high accuracy. (2) The Earth may have acquired its volatiles from a source unlike known meteorite types and one with higher Ar/C and Ar/N ratios. This possibility is plausible since the oxygen isotopic abundances of terrestrial materials is distinct from those of ordinary chondrites, carbonaceous chondrites, or ureilites¹¹. Unfortunately, Ar/C ratios are not known for differentiated objects (for example, achondrites, mesosiderites and the Moon) which have oxygen isotopic patterns analogous to the Earth¹², but which contain very little planetary Ar. (3) It seems unlikely that the relative

respectively, for the whole bodies, whereas the C and N abundances for Mars and Venus are the atmosphere inventories only. The ³⁶Ar/C and ³⁶Ar/N ratios for Mars are higher than those of Earth and meteorites by factors of ~20–4,000, which undoubtedly results because only a small fraction of the C and N acquired by Mars currently resides in its atmosphere⁵. Based on their assumption that volatiles on Mars have abundances like their calculated LL chondrite values, Rasool and Le Sergeant¹ estimated that ~3.5 % of the original C and ~6 % of the original N on Mars would be present in its atmosphere (assuming that all of the original ³⁶Ar is present in the atmosphere). If known meteorite types (Fig. 1) contributed the volatile elements to Mars, however, these values must be lowered, and only ~0.05 % of the original C and ~0.3 % of the original N on Mars would be present in its atmosphere. These values are consistent with the suggestion that large amounts of carbonates may reside in surface materials¹⁴, and with the estimate made from the ¹⁵N/¹⁴N ratio measured by Viking that ~10–150 times as much N has been lost to space from the atmosphere of Mars as has been retained¹⁵. It is interesting to note that the N/C ratio varies only little among the different objects listed in Table 1 in spite of variations in ³⁶Ar/N and ³⁶Ar/C by two orders of magnitude and more. Even the N/C ratio for the Mars atmosphere, which

apparently has lost large fractions of C and N by different mechanisms, is within a factor of 2 of the N/C values for the whole Earth and meteorites. The N/C ratio, therefore, is not likely to be diagnostic of any component which may have contributed volatiles to the terrestrial planets.

As discussed above, neither the relative abundances of volatile elements supplied to Mars, nor the fractions of original volatiles which now reside in its atmosphere can be precisely determined from the present data. This statement also applies to conclusions drawn from the measured $^{40}\text{Ar}/^{36}\text{Ar}$ ratios, as neither the $^{36}\text{Ar}/\text{K}$ or K/C ratios nor the fraction of ^{40}Ar degassed into the Martian atmosphere are known. It has been suggested that the high $^{40}\text{Ar}/^{36}\text{Ar}$ ratio for the Mars atmosphere ($2,750 \pm 500$ (ref. 5)) compared with the Earth's atmosphere (296) may be due to a higher K content and $\text{K}/^{36}\text{Ar}$ ratio for Mars compared with the Earth, or that ^{36}Ar has escaped from Mars^{16,17}. Fanale¹⁴ has suggested that both Earth and Mars received a K content similar to chondrites and that Mars received an ^{36}Ar content at least as high as Earth's. The K/C , K/N , $^{36}\text{Ar}/\text{C}$ and $^{36}\text{Ar}/\text{K}$ ratios for Earth and meteorites (Table 1) show considerable variation, however, and suggest that appreciable fractionation may have occurred in the $^{36}\text{Ar}/\text{K}$ ratio in the inner Solar System. Therefore this ratio cannot be assumed arbitrarily for Mars. The possibility of subsequent loss of ^{36}Ar from the Mars atmosphere^{16, 17}, and the uncertainty in the relation between early catastrophic degassing of ^{36}Ar and continuous degassing of ^{40}Ar (see, for example, ref. 14) add additional variables to any interpretation of the $^{40}\text{Ar}/^{36}\text{Ar}$ ratio of the Mars atmosphere.

It is also interesting to compare relative abundances of C, N and Ar recently determined in the atmosphere of Venus¹⁸ with those for Mars, meteorites and the Earth (Table 1). For the atmosphere of Venus the N/C ratio is similar to that of the whole Earth and meteorites, and the total amounts of N_2 and Ar in the Venus atmosphere are similar to total amounts in the Earth's atmosphere¹⁸. These observations suggest that large fractions of the C, N and ^{36}Ar on Venus may reside in its atmosphere. As for Mars, however, both the relative abundances of volatiles supplied to Venus and the fractions of original volatile elements which now reside in the atmosphere are unknown. If we assume that volatiles supplied to Venus had $^{36}\text{Ar}/\text{C}$ and $^{36}\text{Ar}/\text{N}$ ratios like the Earth's and that these ratios in the atmosphere of Venus reflect the whole planet inventory, we can calculate $^{40}\text{Ar}/^{36}\text{Ar}$ ratios for Venus of 800 and 1010, respectively, by the following formula and its equivalent for Ar/N :

$$[^{36}\text{Ar}/\text{C}]_{\text{Earth}} + [^{40}\text{Ar}/\text{C}]_{\text{Venus}} = [\text{total Ar}/\text{C}]_{\text{Venus}}$$

Similarly, if we assume volatiles supplied to Venus had $^{36}\text{Ar}/\text{C}$ and $^{36}\text{Ar}/\text{N}$ ratios like CI chondrites, calculated $^{40}\text{Ar}/^{36}\text{Ar}$ ratios for Venus are 16,666 and 16,847 respectively. Calculated $^{40}\text{Ar}/^{36}\text{Ar}$ ratios of ~ 900 would suggest that either Venus has a $\text{K}/^{36}\text{Ar}$ ratio ~ 3 times that of the Earth or that radiogenic ^{40}Ar on Venus has been ~ 3 times more efficiently degassed into the atmosphere. The latter explanation seems to be consistent with the measured high surface temperature on Venus¹⁹ and the recent radar observations of apparent large volcanoes²⁰. Calculated $^{40}\text{Ar}/^{36}\text{Ar}$ ratios of $\sim 16 \times 10^3$, however, would imply either a $\text{K}/^{36}\text{Ar}$ ratio or a ^{40}Ar degassing fraction higher by a factor of ~ 57 on Venus compared to the Earth. It has been estimated that 7–90% of the Earth's total ^{40}Ar (depending on the K content adopted) has been degassed into the atmosphere^{2, 14, 21}. Therefore, to assume that volatiles on Venus have relative abundances like CI chondrites would also require higher $\text{K}/^{36}\text{Ar}$ ratios than either the Earth or CI chondrites (Table 1). The simpler explanation is that volatiles accreted by Venus had relative abundances similar to those on Earth, and that Venus has degassed a higher fraction of its ^{40}Ar . This explanation, however, depends on the assumption that the

$^{36}\text{Ar}/\text{N}/\text{C}$ ratios of the atmosphere of Venus are essentially identical to the whole planet inventory, which may not be true.

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Received 30 June; accepted 4 November 1977.

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Limits to palaeogravity since the late Precambrian

HYPOTHESES involving substantial changes in Earth radius over geological time can be tested by measuring palaeogravity at, or near, the Earth's surface. The measurements are obtained by comparing the pressure–temperature conditions in the upper mantle, disclosed by kimberlite nodules of known age, with the computed pressure–temperature conditions for that time¹. Recently published data for six groups of kimberlites, the earliest from the late Precambrian, are analysed here.

Temperature of formation of pyroxene–garnet bearing nodules can be determined by the diopside geothermometer^{2, 3}. Pressure can be obtained from the alumina content of enstatite⁴. An adjustment of -8×10^8 Pa has been applied to all pressures not corrected for presence of calcium and iron in the system^{5, 6}. An envelope drawn tightly around points on the pressure–temperature plot for each kimberlite suite has been used to measure the uncertainty in the pressure determinations (Fig. 1). Envelopes are elongated, their gradients at relatively low pressures similar to those expected for the palaeogeotherm. Gradients at relatively higher pressures, however, are sometimes steeper. Such 'inflected' geotherms probably indicate some kind of thermal instability in the mantle and so separate calculations have been made for the low and high pressure nodules in each kimberlite suite.

The pressure–temperature conditions in the upper mantle beneath stable continents at the present time have been computed by several workers from measured heat flow and plausible arrangements of heat producing elements and conductivities^{7–9}. The two geotherms plotted in Fig. 1 represent extreme conditions—average continental lithosphere with heat flux 1.0 hfu (ref. 7) and lithosphere beneath Montana with heat flux

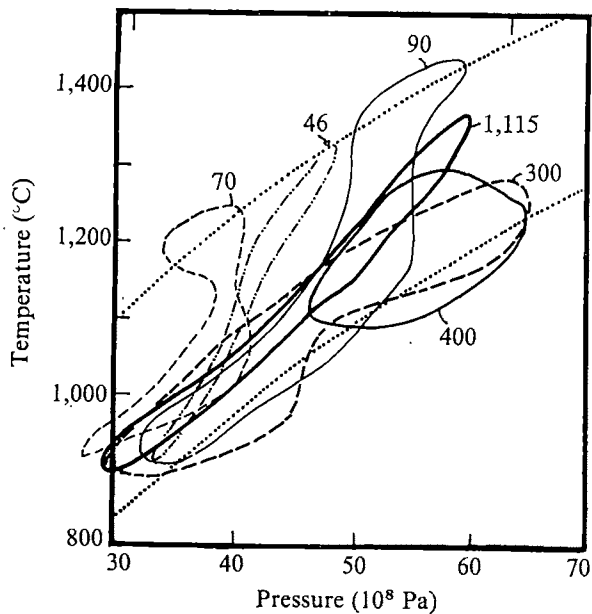


Fig. 1 Pressure-temperature conditions in the upper mantle deduced from ultramafic nodules in six kimberlite suites; ages in Myr BP. Dotted lines are limits to pressure and temperature beneath stable continents at present.

1.2 hfu (ref. 9). Heat production in the past was greater than now, however. For example, 1,100 Myr ago it was 1.2 times the present value¹⁰ and assuming thermal equilibrium the geothermal gradient would have been correspondingly steeper. For each kimberlite suite, therefore, a separate geotherm has been computed (not shown in Fig. 1) assuming a linear decline in heat production over 1,100 Myr.

A maximum limit to palaeogravity can be obtained by dividing the greatest pressure deduced from mineral chemistry ($P_m + p_m$) by the least pressure from the geotherm ($P_g - p_g$), both at the same arbitrarily chosen temperature T_m . Reversing the procedure gives a minimum limit. So

$$g_{max} = [(P_m + p_m)/(P_g - p_g)]g_0$$

and

$$g_{min} = [(P_m - p_m)/(P_g + p_g)]g_0$$

where P is mean pressure, p the uncertainty and g_0 the present value of gravity. The term involving the gradient of the geotherm formerly used¹ may be neglected. Limits derived from the above equations are given in Table 1. A reason for calculating limits rather than mean values for palaeogravity is

the difficulty of selecting a standard geotherm for all continental lithosphere. The difference between pressure-temperature plots for different kimberlites in southern Africa may lie partly in regional variations in the geotherm¹¹. Another reason is the possibility of systematic errors of 10^8 to 10^9 Pa in the pressure estimates derived from mineral chemistry. Nevertheless, the limits are probably more reliable than those derived by using a similar technique on upper mantle peridotites or metamorphic mineralogy¹ for which the geothermal regimes are even more uncertain.

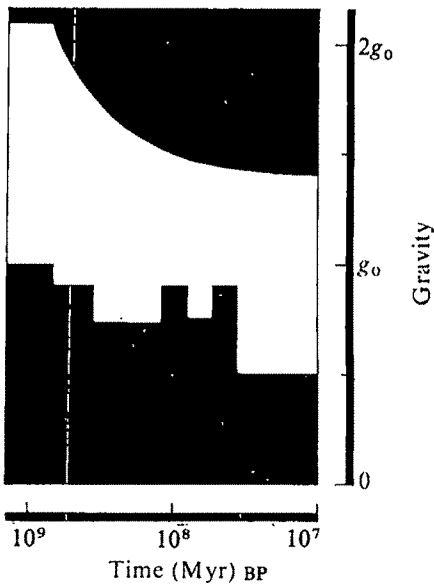


Fig. 2 Limits to palaeogravity over the period 100 Myr to 1,115 Myr. Lower limits come from the kimberlite data in Fig. 1. Upper limits are determined from metamorphic mineralogy (see text). Present gravity at the Earth's surface is g_0 .

The new results show that gravity since the late Precambrian could never have been much less than at present. The lower limit from the Premier Mine is particularly significant; it is fractionally greater than unity and thus lends no support to the most recent version of the contracting Earth hypothesis¹². This hypothesis requires virtually instantaneous core formation by phase change about 2,500 Myr ago, followed by a gradual contraction of Earth radius from 6,671 km (which implies $g = 0.9 g_0$) to the present value of 6,371 km.

The upper limits to palaeogravity obtained are of little use. A more stringent upper limit for the Phanerozoic ($g < 1.4 g_0$) from the non-occurrence of lawsonite in deep sedimentary

Table 1 Limits to palaeogravity derived from the envelopes in Fig. 1

Kimberlite suite	Ref.	Age (Myr)	P_m (10^8 Pa)	p_m (10^8 Pa)	P_g (10^8 Pa)	p_g (10^8 Pa)	$\frac{g_{max}}{g_0}$	$\frac{g_{min}}{g_0}$
Premier Mine	19	1,115	39.0	3.0	24.5	10.0	2.9	1.0
South Africa			55.5	1.5	38.0	15.0	2.5	1.0
Sloan II	20	400	56.7	7.3	42.0	12.0	2.1	0.9
USA								
Udachnaya	21	300	37.5	6.5	27.5	9.0	2.4	0.8
USSR			56.5	6.5	43.0	12.0	2.0	0.9
Kimberley	3,22,23	90	39.7	3.7	31.0	11.0	2.2	0.9
South Africa			52.0	3.0	57.0	14.0	1.3	0.7
South West	24	70	38.0	3.0	35.5	9.5	1.6	0.8
Africa			37.0	3.0	48.0	10.0	1.1	0.6
Montana	25	46	36.5	0.5	32.0	8.0	1.5	0.9
USA			47.0	1.0	59.0	13.5	1.1	0.6

The top line of data for each kimberlite suite relates to low pressures and temperatures and is probably the more reliable.

basins, has already been published¹. The limit rises to 2.0 g_0 at the end of the Precambrian when allowance is made for increased continental heat production. It compares with a Proterozoic maximum of 2.1 g_0 , using a compromise aluminium silicate triple point¹³ ($P_m = 4.8 \times 10^8$ Pa, $p_m = 1 \times 10^8$ Pa, $T_m = 575^\circ\text{C}$, $P_g = P_m$, $p_g = 2.0 \times 10^8$ Pa) and the equations given above. These upper limits flatly contradict rapid Earth expansion models¹⁴⁻¹⁶ unless mass increases with radius. Rapid expansion models require Earth radius to have been 4,500 km in the Carboniferous, 300 Myr ago, which gives $g = 2 g_0$. Slow Earth expansion^{17, 18} is still possible, however.

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Received 4 November; accepted 24 November 1977.

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Phytoplankton biology of a thermal front in the Celtic Sea

SEASONAL thermal fronts which occur in coastal waters are typified by sharp lateral gradients of temperature at the sea surface resulting from a surface outcropping of the thermocline. The frontal regions are areas of considerable physical and biological activity; in the case of the fronts in the Celtic and Irish Seas this increase in biological activity is reflected in marked increases of chlorophyll *a* at the sea surface¹. Considerable data on the spatial distribution of physical and chemical characteristics of the water and chlorophyll *a* concentrations in the vicinity of these and other fronts are now available²⁻⁵. But, few observations have been made either on the temporal variability of these characteristics or on the more detailed distribution of phytoplankton productivity. During a survey of the Celtic Sea in September 1976 we made consecutive crossings of the frontal region in order to investigate the small-scale distributions of chlorophyll *a* and primary productivity and their interrelationships with selected physical and chemical variables.

Temperature, salinity and concentrations of dissolved silicate and chlorophyll *a* were recorded continuously along the ship's track from water pumped through a port in the ship's hull at a depth of 2 m (ref. 2). Single observations of productivity were determined from phytoplankton samples also taken from this source. The productivity was estimated by the standard ¹⁴C uptake method⁶, samples being incubated for 3 h under a light intensity of 6 kl. The samples were cooled by pumping surface seawater through the incubator. During the first two transects of

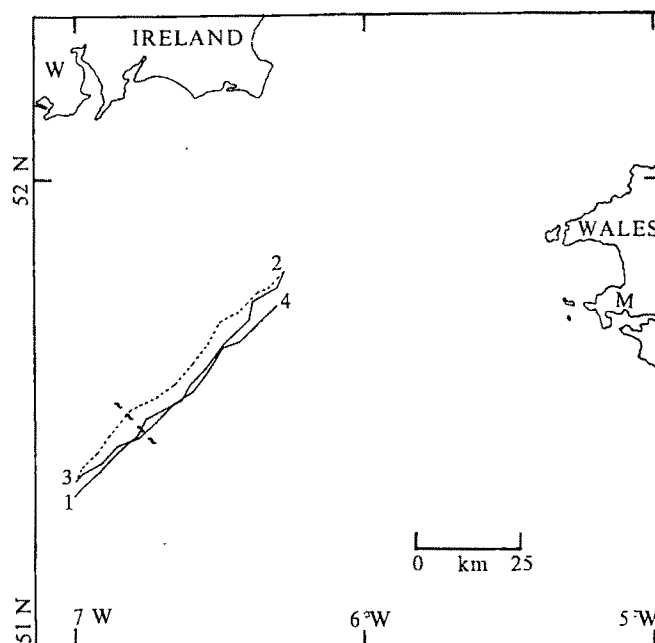


Fig. 1 Location of transects of Celtic Sea thermal front on 8 September 1976. ~~, Approximate position of main frontal boundary. Position 1, start of first transect; position 2, end of first transect and start of second; position 3, end of second transect and start of third; position 4, end of third transect. W, Waterford; M, Milford Haven.

the front, marked increases in productivity and chlorophyll were found to be associated with zones of increased lateral temperature gradient at the sea surface. In the third and final transect, however, this association between the physical and biological properties was masked by the development of sharp local increases in the magnitude of the biological characteristics. These local increases seemed to result from the greater degree of wind mixing experienced on this transect.

Continuous on-line analysis of the physical properties and the dissolved constituents along a transect approximately parallel to the tidal stream (Fig. 1) provided a detailed characterisation of the frontal structure. The essential physical features of the initial and final traverses are summarised in Figs 2 and 3 respectively, and the mean characteristics of the identified water types are listed in Table 1.

In the initial transect, waters exhibiting uniform characteristics typical of supra-thermocline waters in this region (type A) were terminated by a sharp thermal gradient ($\sim 1^\circ\text{C mile}^{-1}$) and chemogradient ($\sim 75 \mu\text{g Si l}^{-1} \text{ mile}^{-1}$). Adjacent to the front a pronounced temperature minimum (the lowest recorded along the transect), with associated salinity minimum and nutrient maxima, indicated a narrow band of water with a significant upwelled component⁷. Between the main frontal features and the major body of cool water (C) lay a lens of warm water (B) bounded by minor but distinct thermal gradients, suggesting a zone of less vigorous mixing in the frontal circulation leading to localised surface heating.

A gradual deterioration in weather conditions accompanied subsequent transects. The essential hydrographic features observed in transect 1 were also evident in the second transect, although in general the boundaries were less well demarcated. Despite the

Table 1 Mean characteristics of the water types depicted in Figs 2 and 3

Water type	Salinity (‰)	Temperature (°C)	Silicate concentration ($\mu\text{g l}^{-1}$)
A (Fig. 2)	35.10	17.50	26
B	34.88	15.70	165
C	34.90	15.25	185
A (Fig. 3)	35.05	17.65	20
D	35.15	16.70	125
E	34.85	15.80	200
F	34.90	15.20	220

increase in the wind-induced mixing, in the final transect the major thermal discontinuity, together with its boundaries of warm and upwelled water, was still prominent. Three additional distinct water types (D, E, F), demarcated by thermal, halo- and chemo- gradients, were evident in a direction away from the major discontinuity. Although the average temperature and salinity of water types E and F were similar to those of the corresponding water types B and C in the previous two transects, average silicate concentrations had increased significantly.

The chlorophyll *a* and productivity records associated with these transects are illustrated. Reference to Fig. 2 clearly demonstrates that the complementation of waters of inherently different properties induces fertility¹. Low chlorophyll *a* concentrations prevailed throughout the stratified waters on the warm side of the major thermal boundary and within the main water bodies of B and C, while chlorophyll peaks occurred at the thermal discontinuities. The fertilisation effect is further emphasised by the magnitude of the chlorophyll peaks at the discontinuities. Low chlorophyll *a* concentrations in the central body of the upwelled water increased towards the bounding thermal fronts. The increase in a direction towards the major temperature gradient was significantly less marked, however, reflecting the reduced degree of mixing between the contrasting water types adjacent to the major lateral density gradient. Maximum chlorophyll *a* concentrations occurred in the minor density gradient between water types B and C where a greater degree of lateral mixing was possible.

In the final transect chlorophyll *a* concentrations exhibited a marked spatial variability (Fig. 3). In contrast to the initial

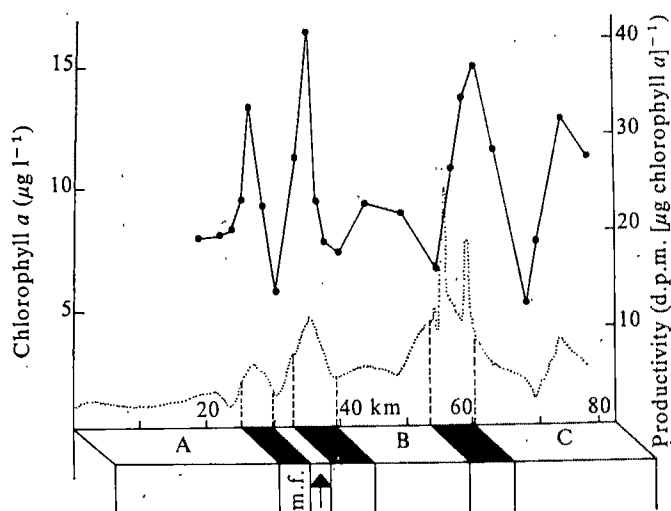


Fig. 2 The physical structure, chlorophyll *a* and productivity records observed during transect 1 across the front 0530 to 0930 8 September 1976. Abscissa denotes distance along the transect. Solid line represents productivity; dotted line represents chlorophyll concentration. Areas of sharp temperature gradient (■), the major front (m.f.), the upwelling zone (↑) and distinct water types are depicted.

transect, high concentrations not only occurred at the boundaries of the various water types, but also within them. Chlorophyll patches, approximately 0.75 miles across and containing up to $15 \mu\text{g l}^{-1}$, existed within water type E and persisted across the discontinuity into water type F. The chlorophyll patchiness coincided with noise on the temperature record indicating a causal relationship between patchiness and wind induced mixing within the bounds of the thermal discontinuities.

The productivity data, based on discrete samples, were less comprehensive. Nevertheless, the initial transect, which was preceded by a period of calm, depicted a distinct pattern of biological activity associated with the main physical features of the frontal system. A positive correlation between productivity and chlorophyll was clearly evident, with increases in productivity being particularly marked at the discontinuities. This relationship is associated with a later stage of development in frontal dynamics

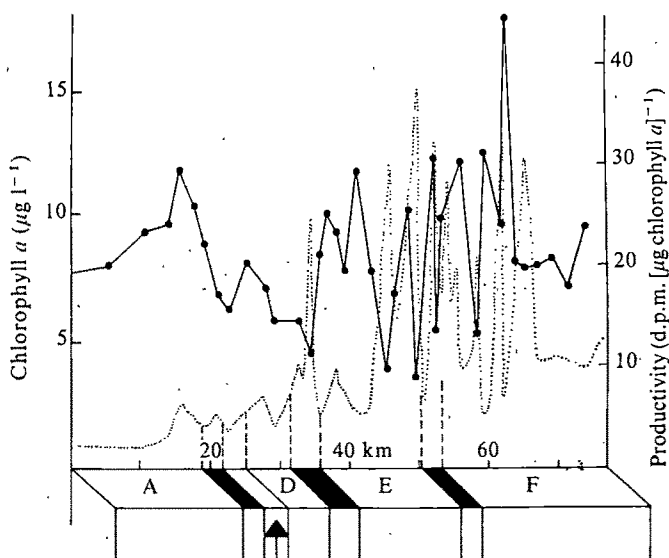


Fig. 3 The physical structure, chlorophyll *a* and productivity records observed during transect 3 across the front 1330 to 1720 8 September 1976. Abscissa denotes distance along the transect. Solid line represents productivity; dotted line represents chlorophyll concentration. Areas of sharp temperature gradient (■), the major front (m.f.), the upwelling zone (↑) and distinct water types are depicted.

during a period of stability. Sufficient time has elapsed for increases in productivity arising from the admixing of the contrasting water types to be reflected in an increase in phytoplankton stock, resulting in the observed positive correlation. For this condition to occur, it is clear that sufficient nutrients must remain in the mixed water in order to sustain a high level of production; also increases in phytoplankton must be in excess of losses from advection, diffusion and grazing. The corresponding early stage in the development of the dynamics will be represented by inverse correlations between these two characteristics: insufficient time will have elapsed for an increase in productivity resulting from admixing of water to be reflected in increases of the stock of phytoplankton.

In subsequent transects, wind induced mixing led to a rapidly evolving pattern of phytoplankton productivity and growth, resulting in a marked increase in the spatial variability of these parameters. The magnitude of the transient features of the biological characteristics was comparable to those observed earlier at the discontinuities. The spatial variability was especially evident in the cool waters of the frontal region in the final transect where an inverse relationship between productivity and chlorophyll was apparent. But, the increased mixing and spatial variability indicate that this relationship was not a reversion to an early stage in the sequential development of phytoplankton dynamics in a frontal region. Further support for this contention is available in the rate of evolution of the chlorophyll patches. Division time of phytoplankton in the sea averages 24 h^8 , whereas the chlorophyll transects showed at times a doubling of concentration within 6 h. The time scale of our observations therefore suggests that the development of the marked spatial variability in chlorophyll distribution was predominantly a consequence of physical aggregation and the outcropping of sub-surface concentrations of phytoplankton.

We thank the Captain and crew of the R.V. Prince Madog for their cooperation and Mr P. Johnston for his assistance with the productivity estimations.

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Received 22 August; accepted 2 November 1977.

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A photoaffinity-labelled insect sex pheromone for the moth *Antheraea polyphemus*

SINCE the isolation of the first insect sex pheromone, bombykol, from the female silkworm *Bombyx mori*¹ and its full identification by synthesis as (10E, 12Z)-10,12-hexadecadienol^{2,3}, the number of characterised pheromones has increased dramatically⁴⁻⁷. The basic question of pheromone-receptor interactions remain unanswered, however. To devise a method which could be used to study such problems, it was necessary to modify the natural pheromone in such a way as to specifically label the binding site of its chemoreceptor cell. We chose to convert the acetate group, a common feature in Lepidoteran pheromones⁴⁻⁷, into a diazoacetate derivative. Information about the active site of the pheromone receptor cell can then be obtained by reacting it irreversibly with a carbene derived from the diazoacetate group. In this procedure, the modified pheromone is radioactively labelled, and since the carbene is generated photolytically, the process is called photoaffinity labelling^{8,9}. We have used electrophysiological techniques as a very sensitive and rapid bioassay method to check the activity of the modified pheromone. We recorded the responses from single receptor cells and evaluated the changes in receptor potential and nerve impulses activity accompanying exposure of the antennae to a stream of pheromone¹⁰⁻¹². We describe here experiments showing that the diazoacetyl derivative of the pheromone acetate (VII) meets the requirements of a photoaffinity label for the study of the receptor.

Fig. 1 Synthesis of the pheromone (6E, 11Z)-6, 11-hexadecadienyl acetate (VII) and its diazoacetate.

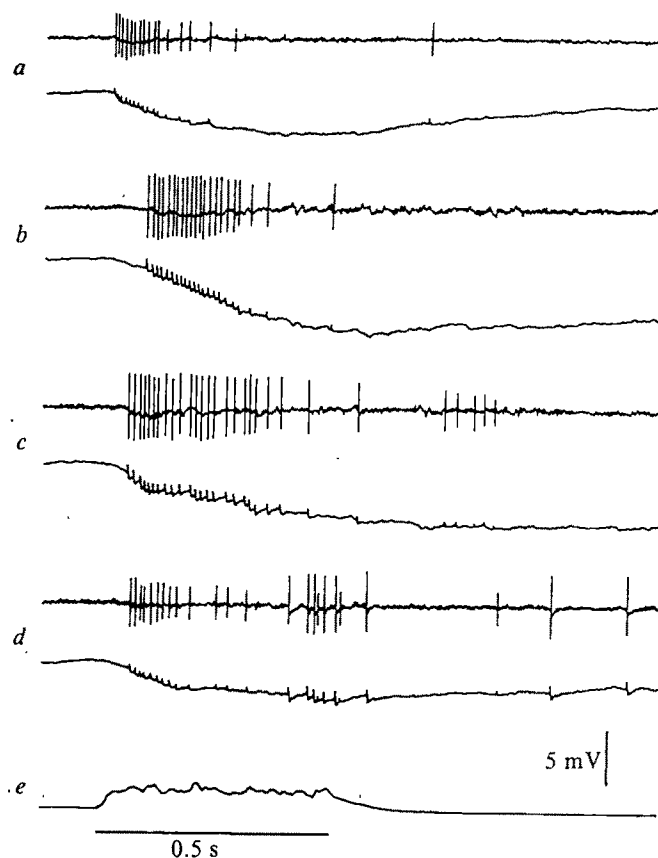
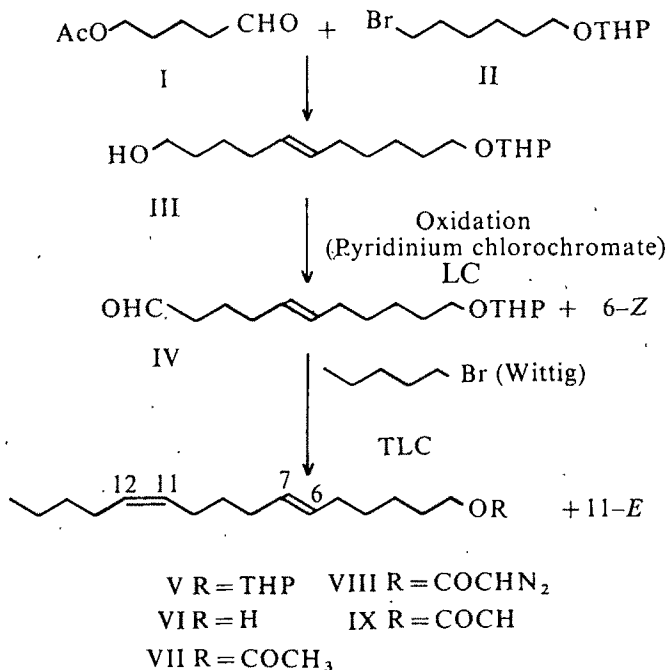


Fig. 2 Electrophysiological recordings from a single olfactory hair which is innervated by two receptor cells. *a*, One cell produces small nerve impulses and responds to (6E, 11Z)-6, 11-hexadecadienyl aldehyde 10^{-3} μg . *b*, The other cell fires large nerve impulses and is sensitive to (6E, 11Z)-6, 11-hexadecadienyl acetate 10^{-3} μg . *c*, The photoaffinity label (6E, 11Z)-6, 11-diazoacetate (DAC) at 10^{-2} μg excites only the 'acetate cell' (large impulses). The stimulus strength is given as the amount of each compound on a 1-cm² filter paper. *d*, The 'aldehyde cell' (small impulses) responds after irradiation of DAC with ultraviolet light (256 nm, 1 min on each side of the filter paper). The 'acetate cell' fires only very few impulses. Probably, most of the diazoacetate is converted into the aldehyde. *a-d*, Each response is given as a d.c. recording (lower traces) showing the receptor potential with superimposed nerve impulses, and as a.c. recording (upper trace) showing the enlarged nerve impulses. *e*, Response of a thermistor indicating the air-stream velocity at the antenna. The 5mV calibration refers to d.c. signals.

The sex attractants of the wild silkworm *Antheraea polyphemus* (Cramer), a saturniid distributed throughout North America, have been characterised as (6E, 11Z)-6,11-hexadecadienyl acetate VII and its corresponding aldehyde by microchemical reactions, single cell recordings, and synthesis¹³. A 9:1 mixture of the acetate and aldehyde elicited strongest attraction to wild males in field tests.

The pheromones and derivatives were synthesised as outlined in Fig. 1. A Wittig reaction between acetoxy aldehyde (I) and tetrahydropyranyl (THP) derivative II of bromohexanol in ether -45°C for 80 min, followed by addition of ethanol, led to concomitant deacetylation to give a mixture of 6E-alcohol III and the 6Z isomer. The mixture was oxidised with pyridinium chlorochromate¹⁴ to the aldehydes which were separated by preparative liquid chromatography (LC) (Waters Prep LC500, 11% ether in hexane) using a silica gel column impregnated with 10% silver nitrate¹⁵. A second Wittig reaction between 6E aldehyde IV and bromopentane in ether at -45°C for 80 min followed by quenching with water, gave a 60:40 mixture of 11Z (V) and 11E THP ethers which were separated by preparative thin layer chromatography (TLC) on silver nitrate impregnated plates. Hydrolysis of the desired 6E, 11Z-THP ether (6% overall yield from acetate I) gave the alcohol VI which was

acetylated to pheromone VII. Reaction of alcohol VI with the *p*-toluenesulphonylhydrazide of glyoxylic acid chloride and triethylamine gave the diazoacetate VIII, with ultraviolet absorption bands (in hexane) of: 243 nm (10,000), 225 (8,900), 203 (12,700). Purity of final products and double bond isomers, except for the diazoacetate, was checked by gas chromatography.

Photolysis of the diazo group in diazo esters, for example VIII, affords the divalent carbene IX which is known to insert into nearby C-H, O-H, and N-H bonds^{8,9,16,17}. The diazoacetate VIII was relatively stable to sunlight; only 23% underwent photolysis when 60 µg in 3 ml hexane was exposed to sunlight for 60 min. But the same amount was totally photolysed after 10 s in a 254 nm Rayonet with 16 tubes.

It is now well-known that slight modifications in the structures of pheromones, for example, isomerism of double bonds or, especially, modification of terminal functions, lead to a drastic (10^{-1} to 10^{-3}) decrease in activity; also it is known that with certain insects the isomeric ratio of *Z/E* plays a critical part in pheromone reception^{18,19}. It was therefore gratifying to find that, when the 6*E*,11*Z*-diazoacetate VIII was submitted to electrophysiological tests (Fig. 2), it retained 10% of the activity of the natural pheromone VII and it elicited response of the acetate receptor cell dendrites but not of the aldehyde-sensitive cells. The 10% retention of activity has provided the incentive for further radiolabelled photoaffinity studies and detection of pheromone receptors.

Furthermore, electrophysiological experiments showed that neither *Antheraea roylei* nor *Bombyx mori* responded to the diazoacetate VIII (or acetate VII), thus indicating that the diazoacetate is not reacting with the *polyphemus* receptor cell in a random nonspecific manner. Interestingly, when a 1 cm² filter paper treated with 10^{-2} or 10^{-1} µg of the diazoacetate was irradiated for 1 min by an ultraviolet lamp, the aldehyde-sensitive cells responded but the acetate-sensitive cells did not.

It is possible that, in these conditions, the carbene undergoes a Wolff rearrangement to a ketene^{17,20} and that the ketene is further photolysed to the aldehyde²¹, the minor component¹³ of the natural pheromone.

Our results show that the diazoacetate equivalent of natural pheromones containing acetate groups; coupled with electrophysiological tests, could offer a promising lead to the study of pheromone receptors in insects.

This work was supported by USNIH (grant AI 10187).

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Infection of human pancreatic beta cell cultures with mumps virus

THE development of a diabetes-like syndrome in virus-infected animals, particularly in certain strains of mice infected with the M-variant of encephalomyocarditis (EMC) virus has recently been demonstrated^{1,2}. The possibility that viruses might also cause diabetes mellitus in humans, particularly in juveniles, has been suggested periodically since the turn of the century^{3–6}. The evidence, however, is largely circumstantial and comes from case reports showing a temporal relationship between the onset of certain viral infections, particularly mumps, and the subsequent development of diabetes. Although it has been known for some time that pancreatitis may be a complication of mumps^{7–10}, specific involvement of the beta cells has never been demonstrated. Since it is not feasible to obtain pancreatic biopsies during the course of viral infections, we initiated experiments to determine if human pancreatic beta cells grown in culture were susceptible to mumps virus. We have used a double-label antibody technique, using fluorescein labelled anti-mumps antibody and rhodamine labelled anti-insulin antibody, and we show here for the first time that human beta cells can be infected with mumps virus.

Human pancreas was obtained at autopsy, usually within 12 h after death, from subjects aged 3 d to 46 yr, from the Division of Transplantation Surgery, Naval Medical Research Institute, Bethesda, and from the Department of Pathology, Children's Hospital, District of Columbia. Specimens were minced, washed in phosphate-buffered saline (PBS) and digested at 37 °C in PBS containing 0.1% collagenase (202 units mg⁻¹), 0.1% trypsin and 1.0% bovine albumin. Pilot experiments with 15 human and 50 non-human primate pancreas showed that this treatment was necessary to prevent gelatinous aggregation of the dispersed cells. The cells then were washed in PBS and resuspended in Ficoll-PBS (23.7% w/v) at a concentration of 2×10^7 cells ml⁻¹. A discontinuous Ficoll-PBS gradient was prepared with layers of 21.0%, 23.7% (containing cells) and 25.0%, and centrifuged at 350g at 4 °C for 20 min. The cells at the interface of the 23.7% and 25.0% layers were then decanted and cultured on glass coverslips in MPNL65/C medium¹¹ with 10% heat-inactivated foetal bovine serum. Immunofluorescent staining of cells collected on a gradient with anti-insulin antibody demonstrated that approximately 1–5% of the cells were beta cells; a 10-fold enrichment over cells not passed through the gradient.

The ABC strain of mumps virus (from Dr Thomas Flanagan) was grown in African green monkey kidney cell line MA-134 (Microbiological Associates). Pancreatic cells that were in culture for 3–4 d were infected with mumps at a virus to total cells ratio of approximately 1.0. At appropriate intervals, coverslips were fixed for 10 min in acetone and stored at 4 °C until reacted with labelled antibodies.

Antiserum to bovine insulin prepared in guinea pigs (from Dr Peter F. Wright) was labelled with tetramethyl rhodamine isothiocyanate (TRITC; Baltimore Biological Laboratories)¹². The specificity of this reagent was determined by reaction of 6-µm cryostat-sectioned, acetone-fixed human pancreas. Only insulin-containing beta cells in the islets stained bright orange (Fig. 1a). The surrounding

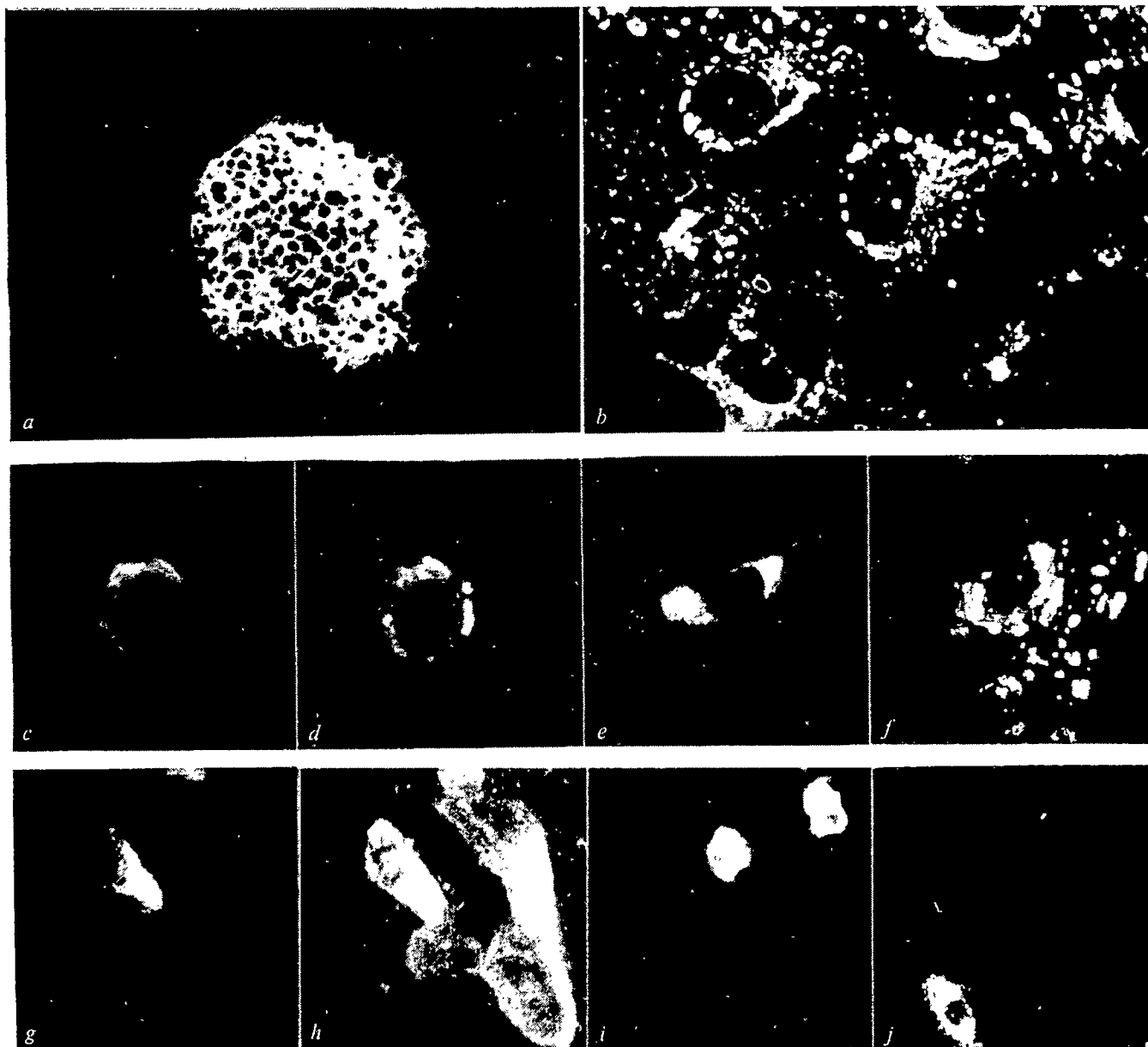


Fig. 1 *a*, Section of human pancreas stained with TRITC-labelled anti-insulin antibody. The photograph was taken with rhodamine filters. *b*, Mumps-infected MA-134 cells stained with FITC-labelled anti-mumps antibody (fluorescein filters). *c*, *e*, *g*, *i*, Human pancreatic cultures infected with mumps virus, stained with TRITC-labelled anti-insulin and FITC-labelled anti-mumps antibodies (rhodamine filters). *d*, *f*, *h*, *j*, Same cultures as above, but photographs taken with fluorescein filters. Magnifications: *b-h*, $\times 2,000$; *a*, *i*, *j*, $\times 800$.

acinar cells did not stain. Absorption of the labelled antibody with purified bovine insulin completely blocked staining (data not shown).

Antiserum to mumps virus, prepared in guinea pigs and labelled with fluorescein isothiocyanate (FITC), was purchased from Microbiological Associates. The specificity of the antiserum was demonstrated by reacting it with mumps-infected MA-134 cells. Mumps antigens stained brilliant apple-green, showed an irregular distribution in the cytoplasm, and seemed more globular than insulin antigens (Fig. 1*b*). Uninfected cells did not stain.

To identify mumps-infected beta cells, coverslips that had been prepared from infected and uninfected pancreatic cultures were stained first with TRITC-labelled anti-insulin antibody, washed and then stained with FITC-labelled anti-mumps antibody. When the order of staining was reversed the rhodamine intensity was slightly diminished, but specificity was not changed.

Coverslips were examined with a Standard Universal Zeiss Microscope illuminated by an HBO 200 mercury lamp. For detection of FITC-labelled anti-mumps antibody, KP490 and

BG12 excitation filters and an LP528 barrier filter were used. For detection of TRITC-labelled anti-insulin antibody, a BP546/9 excitation filter and an LP590 barrier filter were used. Double-stained cells were identified by screening sections first with the rhodamine filters and then with the fluorescein filters.

Human pancreatic cultures were infected with mumps and at various times thereafter stained by the double-label antibody technique. Figure 1*c* and *d* shows a single cell containing both mumps antigen and insulin. When the rhodamine filters were used to examine the cell (Fig. 1*c*), a diffuse homogeneous orange colour, distributed throughout the cytoplasm, was observed. When the same cell (Fig. 1*d*) was examined using fluorescein filters, a brilliant apple-green colour was seen in the cytoplasm. Neither mumps nor insulin was detected in the nucleus. Similarly, the cell in Fig. 1*e* was identified as a beta cell by its orange appearance when viewed with rhodamine filters and as a mumps-infected cell by its green appearance (Fig. 1*f*) when viewed with fluorescein filters. The irregular distribution and globular appearance of the mumps antigen

is very similar to that seen in infected MA-134 cells (Fig. 1b). In addition, Fig. 1f shows mumps antigens in adjacent cells, but the lack of fluorescence in the corresponding positions in Fig. 1e, when rhodamine filters were used, indicates that these cells do not contain insulin. This is even more apparent by contrasting Fig. 1g and h, which show a single beta cell and a portion of a second beta cell (1g) infected with mumps (h) and several non-insulin-containing epithelioid cells also infected with mumps (h). Thus, by use of the double-label antibody technique it is possible to identify infected, insulin-containing, beta cells in a population consisting predominantly of infected non-beta cells. This technique also can be used to detect uninfected beta cells in a field of infected, non-insulin-containing cells. When the rhodamine filters were used, the two cells in Fig. 1i stained bright orange, indicating that they were beta cells; the lack of colour in corresponding positions in Fig. 1j, when the fluorescein filters were used, showed that these beta cells were not infected with mumps. The presence of green fluorescence in the lower left corner of Fig. 1j showed that this cell was infected with mumps, but the lack of orange fluorescence in the corresponding position in Fig. 1i indicated that it was not a beta cell.

To study in more detail the effect of mumps virus replication on pancreatic cell survival, cultures prepared from a single human pancreas were examined daily for 6 d. The percentage of beta cells and non-beta cells that became infected was evaluated by the double-label antibody technique. The data in Fig. 2 show that at 2 d after infection, 32% of the beta cells and 50% of the non-beta cells had become infected with mumps. At 3 d, approximately equal proportions (75%) of beta and non-beta cells

were infected. Virus titres in the cultures increased from $10^{1.2}$ plaque-forming units (PFU) per culture at 24 h after infection to $10^{5.8}$ PFU at 72 h. The infectious titre declined over the next 3 d. The ratio of the number of beta cells in infected cultures to the number of beta cells in uninfected cultures declined from 1.0 at 24 h after infection to 0.1 at 6 d after infection. The highly lytic nature of the mumps infection points to beta cell death as the most likely explanation for this decrease rather than virus-induced degranulation. A similar decline in the number of non-insulin-containing cells in infected cultures also was observed.

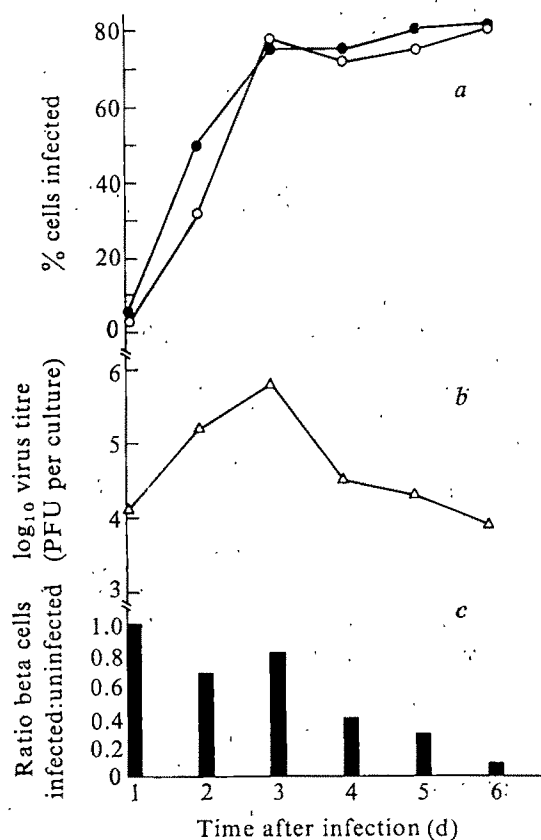
In all, pancreatic cultures from seven subjects were examined for their susceptibility to mumps virus. Patients were of both sexes, and ranged in age from 3 d to 46 yr. The proportion of beta cells in these cultures, determined by staining with TRITC-labelled anti-insulin antibody, ranged from 1–5%. By the third day after infection, 60–90% of the beta cells in these cultures contained mumps antigens. Similarly, 60–95% of the non-insulin containing cells in the same cultures also contained mumps antigens.

By use of the double-label antibody technique, we have shown that human beta cells are susceptible to mumps virus. Moreover, by this technique we have shown that it is possible to identify infected beta cells in a population which contains predominantly (>95%) non-beta cells. In our experiments, beta cells from all seven human pancreas examined seemed to be susceptible to infection with the ABC strain of mumps. We have been able to show that beta cells from African green and rhesus monkeys also are susceptible to *in vitro* infection with mumps virus (data not shown). It must be emphasised, however, that these experiments by no means prove that mumps can produce diabetes. It remains to be determined whether the *in vitro* conditions may have altered the beta cells in such a way as to make them susceptible to a variety of viruses to which they would not have been susceptible *in vivo*. If future experiments show that beta cells grown in culture are restrictive to the replication of certain viruses, but allow the replication of other viruses, then this *in vitro* approach might serve as a useful screening technique for identifying viruses that have a tropism for human pancreatic beta cells and therefore have diabetogenic potential.

Because of the near universal prevalence of mumps, it is clear that if mumps virus is capable of infecting beta cells *in vivo* and producing diabetes, it must do so only in very special circumstances. That is, a particular variant of mumps virus must be involved and/or the individual who develops mumps must have an unusual, possibly genetically determined, susceptibility to the virus. Indirect evidence that a variant of mumps might be involved comes from epidemiologic studies which showed an increased incidence of juvenile diabetes following only certain mumps epidemics^{4–6}. It may be relevant that in mice, only the M-variant of the EMC virus group produces diabetes and the degree of diabetes varies with the passage history of the virus^{1,2}. Host defence mechanisms may also play an important part in determining whether an infected individual develops diabetes. Ordinarily, these host defences prevent the spread of the virus from the salivary glands to the pancreas. If, however, in unusual circumstances, mumps virus reaches the pancreas, the virus may infect and destroy beta cells. Whether such an infected individual develops diabetes may depend on the number of beta cells destroyed.

Finally, it is known that genetically determined host factors control the susceptibility of murine beta cells to EMC virus infection¹. In humans, the frequency of certain HLA antigens (for example, B8, BW15) is several times greater in insulin-dependent juvenile diabetics than the general population^{13,16}. By use of the double-label antibody technique, it might now be possible to determine if beta

Fig. 2 Infection of human pancreatic cultures. a, Percentage of beta cells infected was determined by staining cultures with TRITC-labelled anti-insulin antibody and FITC-labelled anti-mumps antibody. ○, Beta cells; ●, non-beta cells. b, Viral titres were determined by assay on MA-134 cells and are expressed as plaque-forming units (PFU). c, The number of beta cells in infected as compared with uninfected cultures is expressed as a ratio.



cells from individuals with different HLA types show any difference in susceptibility to viral infections.

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Received 27 July; accepted 14 November 1977.

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Prostaglandins in urine of foetal lambs

ALTHOUGH prostaglandins of the E and F series have been measured in amniotic fluid from several species¹⁻³ their principal site of synthesis remains uncertain. In man, for example, the decidua^{4,5} foetal membranes^{6,7} and myometrium⁸ have all been shown to synthesise prostaglandins *in vitro* and each may contribute to the prostaglandin composition of amniotic fluid. The foetal lung and kidneys secrete considerable volumes of fluid into the amniotic sac^{9,10} and hence may also contribute to the prostaglandin content of amniotic fluid. The levels of prostaglandins in foetal tracheal fluid have been shown to be much lower than those in amniotic fluid¹¹ and it was suggested that foetal urine is a more likely source of amniotic fluid prostaglandins. The foetal kidney is capable of synthesising prostaglandins¹² and there is a growing body of evidence that prostaglandins are intimately involved in renal function¹³⁻¹⁵. We have, therefore, measured the concentrations of PGE, PGF and 13, 14-dihydro-15-keto-prostaglandin F (PGFM) in foetal urine during late pregnancy in sheep and have further determined the osmolality, Na⁺ and K⁺ concentrations of foetal urine and plasma. The concentrations of prostaglandins measured in foetal urine were similar to amniotic fluid levels but significantly greater than those previously reported in plasma and tracheal fluid suggesting that the kidney is a major source of amniotic fluid prostaglandins. The excretions of prostaglandins were related to the rate of urine flow; the excretions of PGF and PGFM were correlated with osmolar and solute-free water clearances and Na⁺ excretion, whereas excretion of PGE was related only to the clearance of solute-free water. Prostaglandins may thus contribute substantially to renal function and volume homeostasis in foetal life.

Four cross-bred sheep were used in this study. At laparotomy between 115 and 120 d of gestation catheters were implanted into a foetal femoral artery, tarsal vein and amniotic sac and also into the foetal urinary bladder using procedures described previously^{16,17}. Daily samples (2 ml) were taken from the femoral artery for measurements of P_{O_2} , P_{CO_2} , pH and haematocrit in whole blood and osmolality, Na⁺ and K⁺ concentrations in plasma. Foetal urine was collected for periods of 1-4 h by placing the free end of the bladder catheter 30 cm below the level of the foetus and allowing urine to drain by gravity. Plasma and urine samples were frozen immediately after collection and stored at -20 °C until analysis. P_{O_2} , P_{CO_2} and pH were measured using a Radiometer blood gas analyser. Osmolalities

Table 1 Prostaglandins in urine from four foetal lambs at 124-144 d gestation

	PGE	PGF	PGFM
Concentration (ng ml ⁻¹)	4.59±0.61 (18)	1.96±0.41* (23)	2.64±0.19† (23)
Excretion (ng min ⁻¹)	2.36±0.31 (18)	0.98±0.19* (23)	1.36±0.18† (23)

Values are means ± s.e.; figures in parenthesis are no. of samples.

* $P < 0.01$ compared to PGE.

† $P < 0.001$ compared to PGE.

were measured by freezing point depression (Advanced Osmometer) and Na⁺ and K⁺ concentrations were measured by atomic absorption spectrophotometry (Pye Unicam). Prostaglandins were measured by specific radioimmunoassays^{2,11}. Statistical differences were calculated using the Student *t* test and correlations were assessed by the method of least squares.

The mean concentrations and excretions of PGE, PGF and PGFM in urine sampled daily from foetuses between 120-144 d of gestation are shown in Table 1. The concentration of PGE was significantly higher than the concentration of PGF ($P < 0.001$) or PGFM ($P < 0.01$). There was no correlation between the concentrations of PGE and PGF but the concentrations of PGF and PGFM were significantly related ($r = -0.67$, $P < 0.001$, $n = 23$). The concentrations of PGE, PGF and PGFM in urine were significantly greater than those previously described in foetal plasma and tracheal fluid (Table 2).

The concentrations and excretions of prostaglandins were not related to the arterial blood gases, haematocrit, or the pH of either blood or urine. The excretion of PGE, but not PGF or PGFM, increased with gestational age ($r = -0.62$, $P < 0.01$, $n = 18$). The ratio of the mean concentrations of PGE and PGF was 2.34 (see Table 1), although the most commonly observed (modal) value was 1.25. The excretions of PGE, PGF and PGFM were significantly related to the rate of urine flow (Table 3). The excretions of PGF and PGFM correlated with osmolar and solute-free water clearances and also with Na⁺ excretion, whereas the excretion of PGE was related only to the clearance of solute-free water.

The relatively high concentrations of prostaglandins in foetal urine strongly suggest that the foetal kidney is an important source of prostaglandins in the foetus and probably a major source of the prostaglandins found in amniotic fluid. The excretion of PGE is similar to its concentration in amniotic fluid implying a high turnover in the amniotic fluid. Excretion of prostaglandins in urine closely reflects biosynthesis by the kidney¹⁸. Prostaglandins enter urine from the medulla at the ascending limb of the loop of Henlé¹⁹ and, in the adult animal, undergo considerable intrarenal metabolism^{19,20}. Even assuming that minimal degradation of prostaglandins occurs within the

Table 2 Prostaglandins (ng ml⁻¹) in plasma urine, amniotic fluid, and tracheal fluid from foetal lambs

	PGE	PGF	PGFM
Urine	4.59±0.61 (18)	1.96±0.41 (23)	2.64±0.19 (23)
Plasma ³	0.34±0.028* (19)	0.177±0.013* (19)	—
Amniotic fluid ¹¹	2.54±0.41† (21)	3.78±0.97 (21)	2.88±0.26 (21)
Tracheal fluid ¹¹	0.96±0.19* (21)	0.33±0.07* (21)	0.77±0.07* (21)

Samples of plasma, urine, amniotic fluid and tracheal fluid were obtained from different foetuses at various times during the 1975-76 sheep season in Oxford. Values are means ± s.e.m. with no. of observations in parentheses.

* $P < 0.001$ compared to prostaglandins in urine.

† $P < 0.01$ compared to prostaglandins in urine.

Table 3 Correlation of prostaglandin excretions (ng min⁻¹) with urine flow, osmolality, osmolar clearance, solute-free water clearance and sodium excretion

	U (ml min ⁻¹)	U _{osm} (mOsm/kg H ₂ O)	C _{osm} (ml min ⁻¹)	C _{H₂O} (ml min ⁻¹)	Na excretion (μEq min ⁻¹)
PGE excretion (n = 18)	+0.46*	NS	NS	+0.58*	NS
PGF excretion (n = 21)	+0.53*	NS	+0.45*	+0.65†	+0.49*
PGFM excretion (n = 21)	+0.86†	NS	+0.74†	+0.80†	+0.44*

Values are Pearson correlation coefficients: U, urine flow; U_{osm}, urine osmolality; C_{osm}, osmolar clearance; C_{H₂O}, solute-free water clearance; NS, not significant.

*P < 0.05.

†P < 0.001.

‡P < 0.01.

foetal kidney the excretion rates in the present study are high compared with the conscious adult rabbit²¹ and anaesthetised dog¹⁸. The foetal lamb kidney can metabolise prostaglandins¹², however, and relatively high levels of PGFM were found in foetal urine. Hence it is possible that the foetal kidney has a greater capacity for prostaglandin biosynthesis than that represented by the direct excretions of PGE and PGF.

The high concentrations of prostaglandins found in urine have important implications for several aspects of renal function in foetal life. Renal prostaglandins are known to modulate renin release, salt and water homeostasis and to potentiate adrenergic nerve activity (for recent reviews see refs 22, 23). It is possible that renal production of PGE may be partly responsible for the hypotonicity of foetal urine and poor concentrating ability of the kidney since PGE is known to antagonise the actions of vasopressin on the collecting duct²⁴ and to increase independently solute-free water formation by an action on the proximal tubule²⁵. This hypothesis is supported by the positive correlation found in the present study between the excretion of PGE and solute-free water clearance in foetal urine. Since prostaglandins may modulate the secretion of renin from the kidney²⁶ they may be responsible for the relatively high renin activity of foetal plasma²⁷. The correlation between the excretion of PGF and osmolar and Na⁺ excretion is consistent with the renin inhibiting actions of PGF_{2a} (ref. 28).

We thank Professors G. S. Dawes and A. C. Turnbull for their encouragement and the use of departmental facilities. This work was supported in part by the MRC. D.W.W. was a Nuffield Dominions Demonstrator. Prostaglandins and antisera to PGF and PGFM were the gifts of Dr K. T. Kirton (Upjohn); antiserum to PGE was a gift of Dr M. Withnall (May and Baker). We are grateful to Dr J. S. Robinson for his comments on this manuscript.

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Received 23 September; accepted 9 November 1977.

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Physical and physiological properties of dry lung surfactant

The phospholipids of pulmonary surfactant¹ cover the respiratory epithelium and because of their unique properties of lowering surface tension, spreading rapidly and sustaining high surface pressures, allow the lungs to expand easily and prevent their collapse. Deficiency of lung surfactant is the major cause of respiratory distress syndrome in premature babies and also contributes to the respiratory problems of paraquat poisoning and cardiac bypass surgery. It has generally been assumed that surfactant, whether in the lamellar bodies or alveoli, is in an aqueous environment and must, therefore, be hydrated. We show here, however, that fully hydrated surfactant is apparently inactive and that surfactant can only be effective as a surface monolayer if the source of the material is 'dry'.

The critical micelle concentration (CMC) of the principal lung surfactant phospholipid, L-α-dipalmitoyl phosphatidylcholine, in water has the extremely low value of $4.6 \pm 0.5 \times 10^{-10}$ M (ref. 2). In consequence the overwhelmingly preferred domain for a single phospholipid molecule in water is in association with other phospholipid molecules. The resulting aggregates, technically referred to as smectic mesophases or liposomes, come to occupy a minimum space in the hostile environment of water. The reason for the low CMC of the molecule is the large and incompatible-in-water hydrocarbon moiety.

A liposome infrastructure³ is characterised by alternating bimolecular sheets of lipid intercalated by aqueous spaces, whereby each and every lipid sheet forms a 'closed' membrane system and where all the hydrocarbon chains are screened from water by the polar head-group region (Fig. 1a). It seems unlikely that this material, in a fully hydrated state, could facilitate a rapid extension of an air/fluid interface by donating surface-active molecules, for example, as occurs during the first inspiratory breath of an infant. Nevertheless there has been a tacit assumption that the lamellar bodies of alveolar type 2 cells are packages of lung surfactant, smectic mesophase in character, in equilibrium with cell water.

We suggest that the lamellar bodies are packages of phospholipids in equilibrium with restricted amounts of water—less than about 15 water molecules per phospholipid, at which proportion the bimolecular sheets of phospholipid are not closed but 'open' and the structure has more of the properties of a water-in-oil system than an oil-in-water system. On release from a cell close to an advancing air/fluid interface the phospholipids would rapidly and easily donate molecules to the interface, since these molecules do not have to surmount the unfavourable hydration barrier (Fig. 1b).

The surfactant used in our experiments was prepared by washing out sheep's lungs with saline, removing the cellular debris by centrifugation, concentrating the fluid by lyophilisation and extracting the complete lipid content by the Folch procedure⁴. The chloroform layer was evaporated to dryness under nitrogen leaving a dry, waxy material, the surfactant. This has been analysed and found to contain all the generally accepted surfactant lipids in normal proportions (J. Harwood, personal communication). In the experiments using wetted surfactant, liposomes were formed by sonicating the dry surfactant in saline. Surface tension measurements were made using a roughened platinum dipping plate (2 cm wide) suspended from a force transducer feeding into a recorder. The liquid was contained in a clean, Teflon trough.

When a particle of dry surfactant was placed on the clean surface of physiological saline the surface tension always fell rapidly to the equilibrium tension of $\sim 24 \text{ dyn cm}^{-1}$ (Fig. 2a). If a similar amount of wet surfactant was added to the surface there was either no effect on surface tension or else it fell very slowly to some variable, intermediate value (Fig. 2b).

In other experiments we rapidly removed as much of a surface monolayer as possible by aspiration with a fine tipped sucker (Fig. 2 at each arrow). When the surface layer had been formed from a particle of dry surfactant the surface tension rose with aspiration and then quickly fell again, within 20 s, showing that more molecules were being donated to the surface. This could be repeated many times, but only as long as there was a particle of surfactant at the surface to act as a molecular reservoir (Fig. 2a). Once the particle was removed further recruitment of molecules to the surface became very slow. When wet surfactant was used and the surface monolayer was aspirated, the surface tension rose and fell again very slowly because few molecules were being recruited to the surface (Fig. 2b).

The effect of dry surfactant on lung expansion was investigated by placing a small particle into the fluid-filled trachea of dead 27-d foetal rabbits (term is 31 d, and the lungs contain little endogenous surfactant at this age). Twenty-one foetuses from six litters were used; 22 alternate foetuses were used as untreated controls. A tracheostomy tube was connected to a closed circuit apparatus constructed to inflate and deflate the lungs and to plot pressure volume curves. Three cycles were performed on each lung at 37°C . Twenty-nine foetuses were subjected to pressures up to $35 \text{ cm H}_2\text{O}$ and 14 foetuses to pressures up to $30 \text{ cm H}_2\text{O}$. There was no difference in body weights and wet lung weights between the treated and control

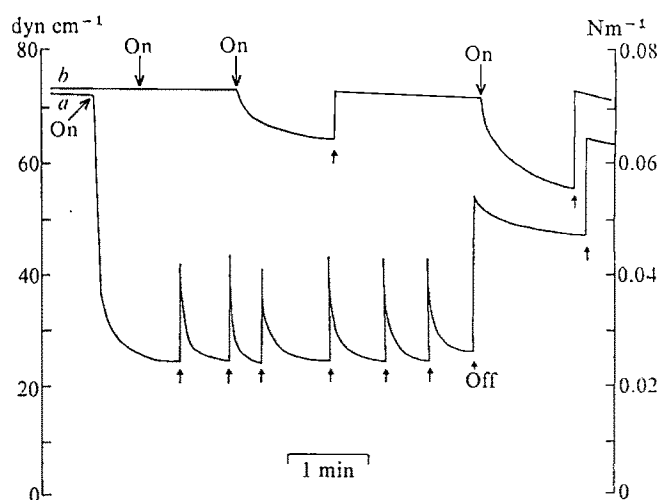


Fig. 2 The surface tension lowering properties of *a*, dry surfactant and *b*, wet surfactant placed on saline. In both instances surfactant was placed on the surface at 'On'. The dry particle was removed from the surface at 'Off'. Upwards-pointing arrows indicate when the surface monolayer was partly removed by aspiration.

foetuses. All the 21 treated lungs expanded at opening pressures below $30 \text{ cm H}_2\text{O}$. Only 6 of the 22 untreated lungs expanded, all with opening pressures above $30 \text{ cm H}_2\text{O}$. The mean volume retained at atmospheric pressure was $0.41 \pm 0.75 \text{ ml}$ (s.e.m.) for the treated lungs and $0.038 \pm 0.012 \text{ ml}$ for the untreated. These differences are statistically significant.

An intact surfactant monolayer is vital to the healthy functioning of the lungs. It allows the lungs to expand with a minimal expenditure of energy and prevents atelectasis in expiration. To be effective a surfactant must maintain the monolayer from the moment of birth. If the surfactant in the lungs is fully hydrated it will not form a surface active layer easily or quickly and will not spread fast enough to maintain an intact surface film. From these experiments we have shown that the active form of surfactant is the 'dry' state. We postulate that the lamellar bodies in the alveolar type 2 cells, which are known to be surfactant stored ready for release into the alveoli, are packages of surfactant in a 'dry' state.

There have been many attempts to treat respiratory distress syndrome in the newborn with surfactant substances as a mist nebulised with water. Not surprisingly this has proved uniformly ineffective. Our results suggest that it may be possible to correct surfactant deficient states with 'dry' surfactant.

This work was supported by the Nuffield Foundation, the MRC and the National Fund for Research into Crippling Diseases. C.J.M. thanks Professor G.S. Dawes for accommodation, facilities and help. We also thank D. J. Klass for advice.

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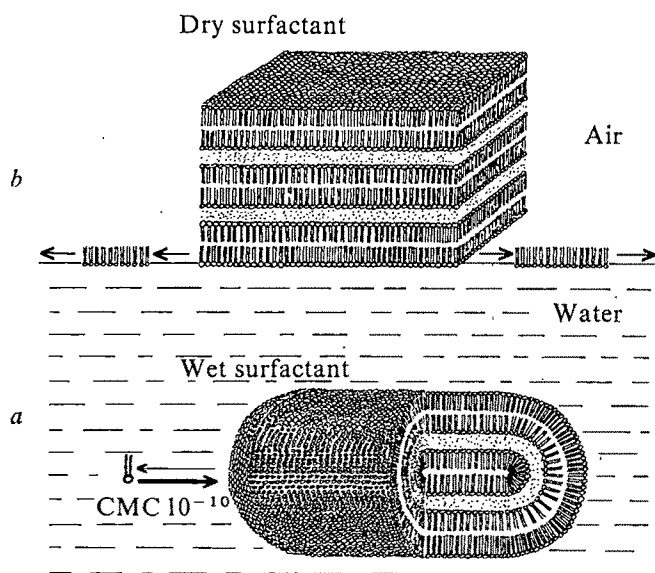
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Fig. 1 The molecular configuration of dry and wet surfactant. *b*, The dry surfactant has open ended layers from which molecules can spread freely to an air/water interface. *a*, In water the surfactant aggregates as a smectic mesophase and few molecules are free to reach the interface.



Vulnerability of methylcholanthrene-induced tumours to immunity aroused by syngeneic foetal cells

It is a well-observed fact that the growth of malignant tumours in experimental animals and in man is often accompanied by the renewed formation of embryonic or foetal substances the synthesis of which would normally have been switched off much earlier in life. This manifestation of anaplasia has often been reviewed¹⁻³. Among such foetal substances are antigens capable of arousing a cell-mediated immunity (CMI) directed both against embryonic cells and also against tumours. This makes it possible to perform experiments which would normally have been prohibited by the fact that the tumours induced by chemical oncogens are almost always antigenically *sui generis*. We present evidence that protection against tumours is secured by immunity to embryo-specific antigens aroused by normal pregnancy or by deliberate inoculation of embryonic cells. This might explain the known association between childbearing and the diminished risk of breast cancer.

A team at this institute had already shown² that the inoculation into CBA mice of irradiated cells isolated from 9-11-d-old CBA embryos 14 d before the subcutaneous injection of 50 µg of 3-methylcholanthrene (MCA) retards the onset and diminishes the final total frequency of tumours. They showed at the same time that when the inoculation of foetal cells was postponed until shortly before the first tumours were expected to arise (about 90 d), the MCA-induced tumours arose more quickly and in more mice. Further investigation (Fig. 1) confirmed the protective power of a foetal inoculum given 14 d before the administration of MCA, and showed again that an inoculum delayed until 14 d after MCA promoted the growth of tumours. The injection of a miscellany of irradiated adult CBA cells 14 d before MCA (under the conditions in which foetal cells are inhibitory) was without effect. In this experiment, however, the injection of adult cells 14 d after MCA caused some tumour promotion. This phenomenon, and the necessity for irradiation of the foetal inoculum if foetal cells are to be immunogenic, are still unexplained.

The balance between tumour promotion and tumour inhibition was now investigated in an experiment in which the ostensibly protective inoculum of foetal cells was administered 7 or 14 d before, or 7 or 14 d after the injection of 50 µg MCA.

The experiment was so arranged that the operation least easy to make uniform was carried out with a single homogeneous

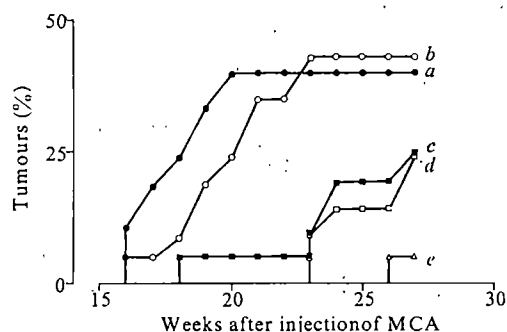


Fig. 1 Cumulative curves of tumour incidence in male CBA mice which, in addition to receiving 50 µg 3-methylcholanthrene (MCA), received inoculation of 1×10^6 foetal or adult cells at various intervals before (—) or after (+): A, foetal cells 14 d after MCA (+14); B, adult cells (+14); C, MCA alone; D, adult cells (—14); E, foetal cells 14 d before MCA (—14). Note protective power of foetal tissues given before (E) but not after (A) MCA. Note also that curves C and D are virtually indistinguishable, showing that adult cells are ineffective under the conditions in which foetal cells confer protection. Adult cells injected 14 d after MCA (B) secure some degree of promotion.

batch of irradiated CBA foetal cells on a single occasion: each of 125 female CBA mice of the same provenance received 1×10^6 irradiated (2,000R) foetal cells belonging to a single batch isolated on the same occasion from 10-11-d-old CBA embryos. The injections of MCA (0.1 ml per mouse 5% MCA in olive oil) were so arranged that each of five groups of 25 mice received their injections of MCA 7 or 14 d before or after, or at the same time as, the injection of foetal cells, at the times indicated in Fig. 2. For logistic reasons, mice receiving MCA alone could not be included, but internal comparisons between the groups show very clearly that inoculation of embryonic cells 7 or 14 d after or at the same time as the injection of MCA was much less effective than the inoculation of foetal cells 7 or 14 d before MCA.

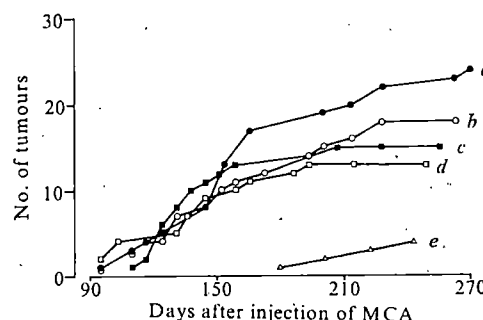


Fig. 2 Cumulative curves of tumour incidence in female CBA mice which, in addition to receiving 50 µg MCA, received inocula of 1×10^6 foetal cells either on the same occasion (0) or at 7 or 14 d before (—) or after (+): A, foetal cells +14; B, foetal cell injection +7; C, foetal cells and MCA simultaneous; D, cell injection —7; E, foetal cells —14. Note relative effectiveness of foetal tissue given before MCA (E, D), compared with the effect of a cellular inoculation at the same time as (C), or after MCA (A, B).

The failure of experiments of therapeutic rather than prophylactic design, and the fine balance between discouragement and promotion of the growth of tumours may perhaps explain the disappointing clinical results of specific tumour therapy. They may also help to account for some unexpected characteristics revealed in the large-scale multi-centre epidemiological studies of the Harvard School of Public Health^{4,5} on the influence of parity on susceptibility to mammary cancer in human beings: the risk of breast cancer decreases with increasing parity, but the principal determining factor is the age of the mother at the birth of her first child; the relatively protective effect of a completed pregnancy before the age of 20 may last for 50 years. The explanation of this phenomenon may be endocrinological, but there is strong evidence that it is immunological: Brawn⁶, Hellstrom⁷ and Simpson and Gautam (personal communication) have shown that pregnancy in mice excites the formation of lymphocytotoxic cells directed against foetus-specific and tumour antigens; Moon⁸, moreover, has shown that rats which have borne litters show an enhanced resistance to the mammary tumours induced by the oral administration of dimethylbenzanthracene. The Harvard collaborative study made it clear that women who bore their first children at the age of 33 or later were more susceptible than childless women to mammary tumours. So far from being inconsistent with an immunological interpretation, this finding is what might have been anticipated from our own experimental results. The effect of an early pregnancy is in a sense simulated by the results of experiments in which immunity is aroused before the application of the oncogenic stimulus; the lessened effectiveness, even increased hazard associated with late pregnancy is simulated by the enhancing effect on tumour growth consistently found when the ostensibly protective inoculation is deferred until after the application of the oncogenic stimulus. The underlying assumption in both situations is that the transformation from normal

to malignant growth occurs very shortly after inoculation of MCA and very early in reproductive life.

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Received 2 September; accepted 21 November 1977.

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H-2 Antigens on a murine lymphoma are associated with additional proteins

THERE have been reports indicating that the normal phenotypic expression of histocompatibility antigens is altered on tumour cells¹, and a disappearance or diminution of H-2 expression on murine tumour cells has been demonstrated^{2,3}. The nature of these changes has so far remained unclear. It has also been shown that H-2D and H-2K antigens are important in T-cell mediated lysis of virus infected or transformed syngeneic cells^{4,5}. One explanation for these phenomena proposes that H-2 antigens are modified by association with additional cell-surface molecules to form a neoantigen or 'altered self'⁶. We report here immunochemical evidence for alteration of H-2 antigens on tumour cells by association with additional proteins.

An oestrogen-induced lymphoma (6C3HED) (obtained from Dr M. Prager, Dallas, Texas) was maintained in syngeneic C3H mice by weekly intraperitoneal transfer of approximately 10^6 tumour cells. We have observed that H-2.23, the private K locus antigen of the H-2^k haplotype, is undetectable on intact 6C3HED lymphoma cells by either cytotoxicity or absorption assays. This is in agreement with the results of Garrido *et al.*⁷. We have also observed, however, that a cell lysate produced by repeatedly freezing and thawing 6C3HED cells completely and specifically blocked the cytotoxic action of anti-H-2.23 on syngeneic normal cells. These data suggest that H-2.23 may be present on 6C3HED cells, but modified in such a way as to be undetectable on the intact tumour cell.

Normal C3H splenocytes and tumour cells were radiolabelled, extracted with 0.5% NP-40, and reacted with either anti-H-2.23 or normal mouse serum. The resulting antigen-antibody complexes were precipitated with Protein A-bearing *Staphylococcus aureus* and analysed by polyacrylamide gel electrophoresis in the presence of sodium dodecyl sulphate (SDS-PAGE).

There was a marked difference in the reactivity of anti-H-2.23 with normal C3H cells and with 6C3HED tumour cells (Fig. 1). Proteins with mobilities corresponding to molecular weights of 45,000 and 12,000 characteristic of detergent-solubilised H-2 and β_2 -microglobulins (β_2 - μ), respectively, were present in both electropherograms. Additional major peaks with mobilities indicative of molecular weights of approximately 70,000 and 55,000 were evident in the 6C3HED cell extract. To establish further that the protein peaks at 45,000 and 12,000 were H-2 and β_2 - μ and to determine whether H-2 antigens on the tumour cells are associated with β_2 - μ , additional experiments were carried out using a rabbit anti-rat β_2 - μ . This antiserum cross reacts with mouse β_2 - μ (ref. 8) and was prepared against homogeneous rat urinary β_2 - μ , which had been shown to give a unique amino acid sequence (M. Poulik, D. Schinnick and O. Smithies, unpublished); the specificity of this antiserum is known⁸. SDS-PAGE analyses of antigens isolated from biosynthetically labelled C3H splenocytes or 6C3HED tumour cells with rabbit anti-rat β_2 - μ are shown in Fig. 2. When tested with normal splenocytes, the anti- β_2 - μ produced peaks characteristic of β_2 - μ and detergent-solubilised H-2. When reacted with tumour cells, this antiserum also

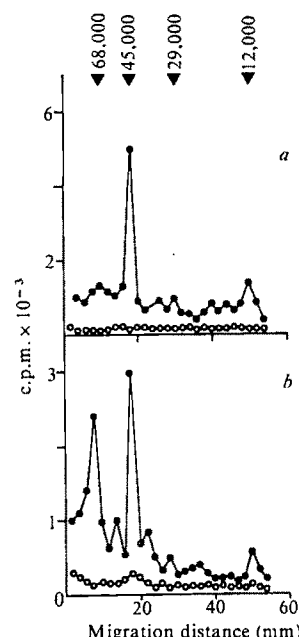


Fig. 1 SDS-polyacrylamide gel electrophoresis of murine lymphoid cell antigens isolated with anti-H-2.23 antiserum (K603). Cells were teased from whole C3H spleens or obtained from the ascites fluid of tumour-bearing mice, washed, counted, and adjusted to a concentration of 5×10^7 viable cells per ml in RPMI 1640 containing $100 \mu\text{Ci ml}^{-1}$ ^3H -leucine and incubated for 5 h at 37°C in a 5% CO_2 -95% air mixture. The cells were then collected, washed, and extracted in 0.5% NP-40 (v/v) in Tris-buffered saline (0.05 M, pH 8.0) for 30 min at 4°C . The extract was centrifuged at $45,000g$ for 15 min and the pellet discarded. Aliquots (100 μl) of the supernatant were incubated with 20 μl of antiserum for 1 h at 37°C , then overnight at 4°C . Indirect immunoprecipitation with 250 μl of 10% v/v heat-killed formalin-fixed *Staphylococcus aureus* (Staph A) (Cowan 1 Strain) and SDS-PAGE on 12.5% gels was carried out as described by Kessler¹⁶. a, N.C3H splenocytes; b, 6C3HED lymphoma cells. ●, Anti-H-2.23; ○, normal mouse serum. Molecular weight standards were bovine serum albumin, ovalbumin, carbonic anhydrase and cytochrome c.

produced the higher molecular weight peaks which were obtained with anti-H-2.23 as well as the H-2 and β_2 - μ peaks. Similar peaks were obtained when anti-H-2.23 and anti- β_2 - μ were reacted with NP-40 eluates of tumour cells which had been radioiodinated with lactoperoxidase. This indicates that the antigens detected were present on the cell surface.

To eliminate the possibility that antibodies reactive with antigens other than β_2 - μ were responsible for the precipitation of additional proteins, the rabbit anti-rat β_2 - μ was prereacted with highly purified rat β_2 - μ or bovine serum albumin (BSA) and then added to a radiolabelled extract of 6C3HED cells. The result of SDS-PAGE analysis of the precipitate is shown in Fig. 2b. The fact that preincubation of the antiserum with purified β_2 - μ reduced all peaks to background level, whereas prereaction with BSA had no effect, strongly suggests that the reactivity of this antiserum with the 6C3HED can be attributed to anti- β_2 - μ antibodies.

Although the reactivities of both anti-H-2 and anti- β_2 - μ antisera with normal and tumour cell extracts were very similar, it was not obvious whether or not they were reactive with the same antigenic structures. Immunoabsorbents (IADS) were therefore prepared by covalent attachment of crude Ig fraction of NRS or rabbit anti-rat β_2 - μ to CNBr-activated Sepharose 4B (ref. 9) and were incubated overnight with radiolabelled extracts of 6C3HED tumour cells. The IADS was then removed by centrifugation and the supernatants immunoprecipitated with anti-H-2.23 or rabbit anti-rat β_2 - μ . SDS-PAGE analyses of these immunoprecipitates are shown in Fig. 3. Precipitation of the H-2, β_2 - μ , and the higher molecular weight peaks by anti-H-2.23 or anti- β_2 - μ was unaffected by previous reaction of the tumour cell extract with the NRS immunoabsorbent. Previous reaction of the tumour cell

extract with the anti- $\beta_2\text{-}\mu$ IADS, however, resulted in removal of all proteins reactive with either anti-H-2.23 or anti- $\beta_2\text{-}\mu$.

There are several possible explanations for the coprecipitation of H-2, $\beta_2\text{-}\mu$, and the higher molecular weight proteins from tumour cell extracts following reaction of both anti- $\beta_2\text{-}\mu$ and anti-H-2 with the lymphoma cells. First, both K603 (anti-H-2.23) and the anti- $\beta_2\text{-}\mu$ sera could be contaminated with additional antibody. We have not established definitely that this is not the case with the anti-H-2.23 antisera, however, since the rabbit anti-rat $\beta_2\text{-}\mu$ was prepared in rabbits against highly purified rat urinary $\beta_2\text{-}\mu$, it seems unlikely that this antiserum was contaminated with additional antibodies reactive with material present only on mouse tumour cells. Second, the apparent association might be an artefact of the extraction procedure, but the proteins are probably not part of a large lipid-detergent micelle since H-2K, H-2D and Ia antigens present in similar extracts of normal C3H splenocytes are easily separated. Complex formation due to disulphide interchange during extraction is also unlikely since identical electropherograms were obtained when the extraction was carried out in the presence of 10 mM iodoacetamide. The appearance of additional proteins in the immunoprecipitate from the tumour cell extracts is not caused by nonspecific binding of proteins to the antigen-antibody complex, since the addition of unlabelled $\beta_2\text{-}\mu$ to the reaction mixture prevented the precipitation of any radiolabelled material. Clearly, then, $\beta_2\text{-}\mu$ protein complexes not present in extracts of normal cells are found in extracts of the 6C3HED tumour cells.

Two explanations exist to account for the relationship between the reactivities of the rabbit anti-rat $\beta_2\text{-}\mu$ and the anti-H-2.23. First, the anti-H-2 antiserum could contain additional antibodies which are reactive with material present on tumour cells but absent from normal cells. These additional antigens, as well as H-2, must be associated with $\beta_2\text{-}\mu$ since prereaction of the 6C3HED cell extracts with the anti- $\beta_2\text{-}\mu$ adsorbent removed all material reactive with anti-H-2. To date, $\beta_2\text{-}\mu$ has been shown to be associated only with H-2 (ref. 10), TLA (ref. 11), and Qa-2 (ref. 12) on the surface of normal cells. A second possibility is that a complex composed of at least one $\beta_2\text{-}\mu$, one H-2 heavy chain and one or two higher molecular weight proteins is formed on the tumour cell. In detergent extracts, this complex precipitates with anti-H-2.23 and anti- $\beta_2\text{-}\mu$ as a single entity. The complex is reduced to its individual protein components when the precipitates are prepared for SDS-PAGE.

At present, we cannot distinguish unequivocally between these two possibilities, but we consider the latter to be the more plausible explanation since four different anti-H-2.23 antisera,

Fig. 2 SDS-PAGE of lymphoid cell antigens isolated with rabbit anti-rat $\beta_2\text{-}\mu$. 100 μl of an extract of either *a*, N.C3H splenocytes or *b*, 6C3HED lymphoma cells was reacted with 20 μl of rabbit anti-rat $\beta_2\text{-}\mu$ antiserum which had been prereacted with either 200 μg of BSA (●) or 100 μg of purified $\beta_2\text{-}\mu$ (○).

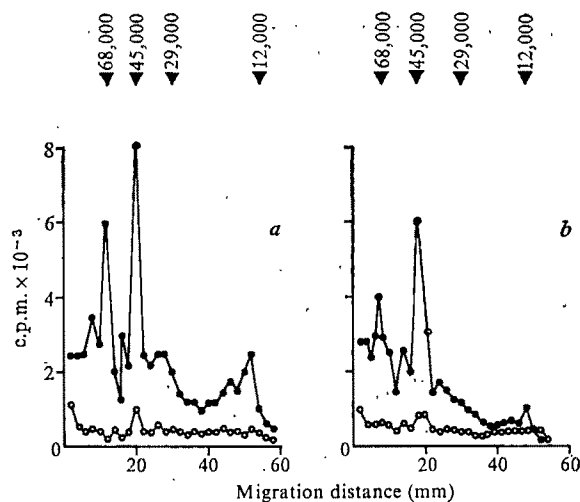
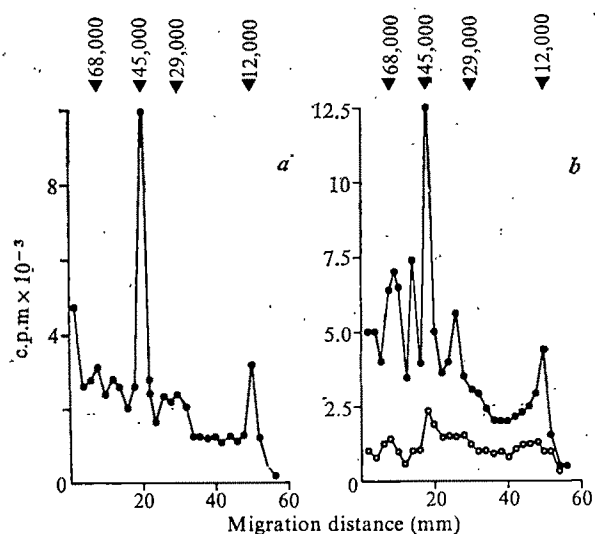


Fig. 3 SDS-PAGE of murine lymphoid cell antigens: effect of pretreatment with immunoadsorbent. Extracts of 6C3HED lymphoma cells were incubated overnight with an immunoadsorbent (100 μl adsorbent per 100 μl extract) prepared by coupling the crude Ig fraction of either rabbit anti-rat $\beta_2\text{-}\mu$ (○) or normal rabbit serum (●) to CNBr-activated Sepharose 4B to a final concentration of ~ 10 mg protein per ml beads. The adsorbent was removed and the supernatant incubated with either *a*, anti-H-2.23 (K603) or *b*, rabbit anti-rat $\beta_2\text{-}\mu$.

including (C3H.OH \times B10.129) against C3H [K603], (B10 \times LP.RIII) against B10.A(2R) [K358], (B10.RIII (7INS) \times DBA/2) against B10.A [K323] and (B10.D2 \times A) against B10A (5R) [D23], gave identical patterns of reactivity in SDS-PAGE analysis of immunoprecipitates of radiolabelled 6C3HED cell extracts. It seems unlikely that all of these antisera would contain precisely the same contaminating antibodies. In addition, preliminary results indicate that anti-H-2 antisera which have been absorbed with normal C3H splenocytes are completely unreactive with extracts of 6C3HED cells. This suggests that the anti-H-2 antisera do not contain additional antibodies which are reactive with materials present only on the tumour cells.

Henning *et al.*¹³ have shown that viral antigens are present in close association with H-2 antigens on the surface of EL-4 leukaemia cells. Furthermore, Fujimoto *et al.*¹⁴ have shown that H-2 is associated with a tumour-associated antigen in the sera of lymphomatous A/J mice and Gooding and Edidin¹⁵ have shown that H-2 is physically linked to an additional antigenic moiety on the surface of teratoma cells. We have presented evidence which strongly suggests that the H-2- $\beta_2\text{-}\mu$ dimer is associated with additional proteins on the surface of 6C3HED lymphoma cells. Furthermore, syngeneic anti-tumour antisera, which are unreactive with normal C3H cells, precipitate the same material from tumour cell extracts that is reactive with anti-H-2.23 (to be published elsewhere). These data suggest that a new complex is formed on the surface of these tumour cells and that this complex contains not only H-2 and $\beta_2\text{-}\mu$ antigenic determinants but also tumour-associated antigens.

We thank Dr Jan Klein for the antisera K603, K358, and K323; rabbit anti-rat $\beta_2\text{-}\mu$ was provided by Dr M. D. Poulik; antiserum D23 was obtained from the Transplantation Immunology Branch of NIAID, Bethesda, Maryland. This work was supported by the USPHS.

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Anti-idiotypic antibodies against anti-vitamin A transporting protein react with prealbumin

IDIOTYPIC determinants on antibodies are those antigenic determinants in one antibody population which are not present in another antibody population from the same animal¹. The restriction of a set of idiotype determinants to antibodies directed against a single antigen suggests that the idiotype determinants may reside in the antibody combining site. In fact, amino acid sequence determinations of antibodies sharing the same idiotype have shown that the variable portions of light chains as well as heavy chains of the various antibodies are very similar if not identical (see ref. 2 for review). These and similar observations have suggested that antibodies against the idiotype determinants (anti-idiotypic antibodies) recognise only a clonotype, that is, antibodies with a given set of variable regions. This seems well founded in as much as antibodies raised in two species against a single antigen only infrequently seem to share idiotype determinants. Since the antibody system is degenerate then some antibodies raised against a protein antigen, called A, which forms part of the protein-protein complex AB, may recognise protein A in a fashion similar to that of the normally interacting protein B. Consequently, the idiotype determinants of some of the antibodies against protein A may have structures in common with protein B. If so, antibodies raised against such idiotype determinants should bind to protein B. To test this assumption we explored the situation with regard to the retinol-binding protein (RBP)-prealbumin protein complex³⁻⁶. The data presented here show that it is possible to raise anti-idiotypic antibodies against anti-RBP and that such antibodies interact with prealbumin.

Human RBP and prealbumin were isolated as described previously³. Various chemical, physical-chemical, and radio-immunological tests revealed that both proteins were free from cross-contamination (for details see ref. 7). Rats were immunised with RBP⁸ and after several booster injections the rats were exsanguinated and the serum collected. Sera from 10 rats were pooled to obtain sufficient amounts for further processing. Antibodies against human RBP were isolated by immunosorbent purification on a Sepharose 4B column to which RBP had been covalently attached⁹. The bound antibodies were desorbed with 0.2 M glycine-HCl buffer pH 2.9. Although RBP which has been subjected to acid does not interact with prealbumin⁹ it was important to ascertain that the RBP antibody preparation did not contain any rat prealbumin. To this end the antibody preparation was labelled¹⁰ with ¹²⁵I and immunoprecipitated with a specific anti-rat prealbumin serum by a sandwich procedure¹¹.

Although this test did not reveal the presence of any prealbumin it is likely that if prealbumin represented less than about 0.002% of the total protein its presence would have escaped detection. Furthermore, heavily overloaded SDS-polyacrylamide gels¹¹ did not reveal any protein zones which could be identified as prealbumin after electrophoresis; more than 98% of the antibody preparation labelled

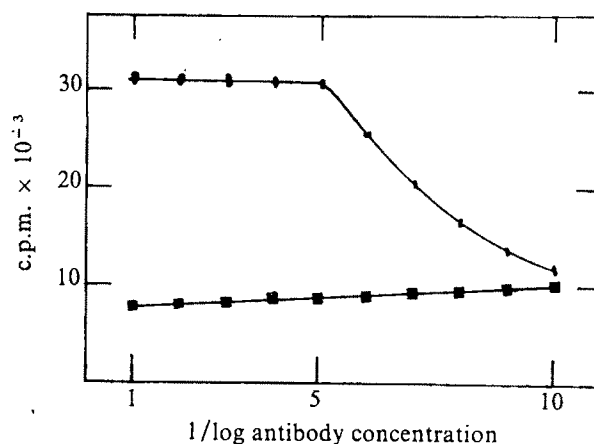


Fig. 1 Binding of anti-idiotypic antibodies against anti-RBP to ¹²⁵I-labelled prealbumin. The anti-idiotypic antibody preparation (initial concentration 2 mg ml⁻¹) was serially diluted and mixed with ¹²⁵I-labelled prealbumin (32,000 c.p.m.). The resulting immune complexes were collected by the use of protein A-containing *Staphylococcus aureus*¹¹. As a control serial dilutions of preimmune rabbit IgG of the same concentration as the anti-idiotypic antibodies was used. ●, Anti-idiotypic antibodies; ■, preimmune IgG.

with ¹²⁵I was precipitated by a rabbit anti-rat IgG serum; and immunoelectrophoresis of the anti-RBP preparation revealed a single precipitin line with a polyvalent rabbit anti-rat serum protein serum. All of these criteria, and others which we shall describe, suggested that the isolated rat anti-RBP antibodies were of high purity.

The specific rat antibodies against RBP were injected into a rabbit which was repeatedly challenged with the antibodies over a period of 3 months. Theoretically, the rabbit antiserum contained antibodies against the common portions of rat immunoglobulin as well as antibodies to the particular idiotype determinants. The IgG of the rabbit serum was isolated on a goat anti-rabbit IgG-Sepharose 4B immunosorbent column. To remove antibodies against the common rat IgG determinants the rabbit IgG fraction was passed over an immunosorbent column of Sepharose 4B containing covalently bound normal rat IgG. Material which passed the column unretarded did not bind to ¹²⁵I-labelled normal rat IgG but reacted with ¹²⁵I-labelled rat antibodies against RBP. Thus, the rabbit antibodies, isolated as described, were apparently specific for idiotype determinants present on rat antibodies against RBP.

To test whether the anti-idiotypic antibodies against anti-RBP would react with human prealbumin, the latter protein was labelled with ¹²⁵I and mixed with serial dilutions of the anti-idiotypic antibodies. The resulting immune complexes were collected as described¹¹. In a control experiment the anti-idiotypic antibodies were replaced by preimmune rabbit IgG. Figure 1 shows that the anti-idiotypic antibodies reacted with prealbumin. In all tests performed the anti-idiotypic antibodies could bind more than 90% of the labelled prealbumin. Preimmune rabbit IgG did not react with prealbumin (Fig. 1), suggesting that the reaction was specific.

The titre of the anti-idiotypic antibodies was low; 1 ml of antiserum could bind 160 ng of human prealbumin and 120 ng of rat prealbumin. One ml of a regular rabbit anti-serum against rat prealbumin bound 150 µg of rat prealbumin but only 10 ng of human prealbumin. These observations show that the immunological cross-reactivity between human and rat prealbumin, as for RBP¹², is low and, thus, the data are consistent with the view that the rabbit anti-anti-RBP serum did not contain any antibodies that had been raised against prealbumin directly.

If the antibodies reactive with prealbumin are genuine

anti-idiotypic antibodies against anti-RBP the binding between prealbumin and these antibodies would be abolished in the presence of antibodies against RBP. To examine this ^{125}I -labelled human prealbumin and anti-idiotypic antibodies against anti-RBP were mixed together with serial dilutions of two rat IgG fractions. One fraction contained rat antibodies against RBP whereas the other fraction comprised only preimmune IgG from the same rats. Figure 2 shows that preimmune rat IgG did not affect the reaction between ^{125}I -labelled prealbumin and the anti-idiotypic antibodies. This reaction was completely inhibited in the presence of rat antibodies against RBP, however.

The same experiment was repeated with a regular rabbit antiserum against rat prealbumin substituting for the anti-idiotypic antiserum. In this case neither rat anti-RBP nor preimmune rat IgG had any effect on the binding of the prealbumin-antibodies to prealbumin (not shown). These data give strong support to the view that it was the anti-idiotypic antibodies against RBP which recognised prealbumin.

Since there is no apparent immunological cross-reactivity between RBP and prealbumin⁸ it seemed likely that the anti-idiotypic antibodies against RBP recognised the RBP-binding site on the prealbumin molecule. If so, RBP should abolish the interaction between the anti-idiotypic antibodies and prealbumin. To examine this various amounts of RBP were added to a mixture of ^{125}I -labelled prealbumin and the anti-idiotypic antibodies. As a control RBP was replaced by soy bean trypsin inhibitor. Figure 3 shows that RBP, but not soy bean trypsin inhibitor, completely abolished the interaction between prealbumin and the anti-idiotypic antibodies. This suggests that the anti-idiotypic antibodies bound to the RBP-binding site on prealbumin.

To explain why anti-idiotypic antibodies raised against anti-RBP interact with prealbumin it seems reasonable to assume that a portion of the antibodies raised against RBP recognises this protein in a way similar to the way prealbumin recognises RBP. Due to the degeneracy of the antibody system, some of the combining sites of the antibodies may in fact be very similar to those structures of

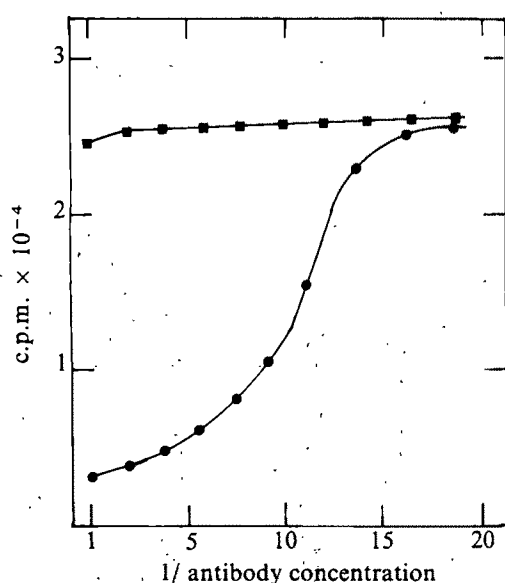


Fig. 2 Competition for binding to the anti-idiotypic antibodies against anti-RBP between prealbumin and rat antibodies against RBP. ^{125}I -labelled prealbumin (30,000 c.p.m.) was mixed with enough anti-idiotypic antibodies to cause 80% binding and serial dilutions of specific rat antibodies against RBP (initial concentration 0.5 mg ml^{-1}). As a control the rat antibodies were replaced by preimmune rat IgG at a concentration identical to that of the RBP antibodies. Immune complexes were precipitated as described before¹¹. ●, Rat antibodies against RBP; ■, pre-immune rat IgG.

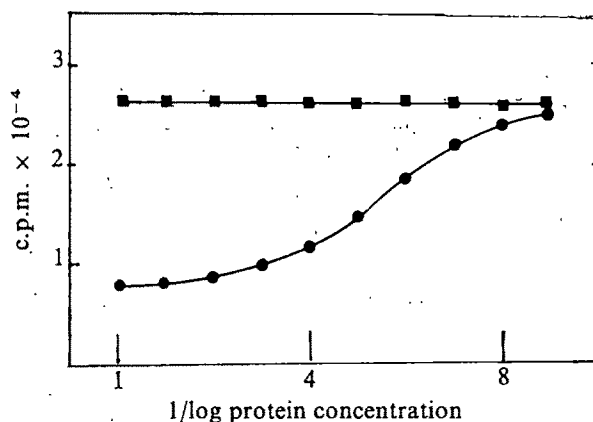


Fig. 3 Competition for binding to prealbumin between RBP and anti-idiotypic antibodies against anti-RBP. ^{125}I -labelled prealbumin (29,000 c.p.m.) was mixed with enough anti-idiotypic antibodies to ascertain 80% binding and serial dilutions of highly purified RBP (initial concentration 0.25 mg ml^{-1}). As a control soy bean trypsin inhibitor was used instead of RBP. Immune complexes were collected as described¹¹. ●, RBP; ■, soy bean trypsin inhibitor.

prealbumin which binds to RBP. If so, a second set of antibodies raised against such antigen-combining sites would cross-react with prealbumin. The reaction with prealbumin should be confined only to that part of the molecule which binds to RBP. This is indeed the finding since RBP competes efficiently with the anti-idiotypic antibodies for binding to prealbumin despite the fact that antibodies raised directly against prealbumin will react with this protein regardless of whether RBP is bound or not⁸.

Only a subset of the anti-idiotypic antibodies would recognise prealbumin since RBP displays multiple antigenic sites⁸. In fact, RBP interacts with a cell-surface receptor with a site that is distinct from the prealbumin-binding site^{4,12}. Another subset of anti-idiotypic antibodies against anti-RBP abolishes the interaction between RBP and the cell surface receptor (K.S. and P.A.P., in preparation).

If generalised, the method described here to raise antibodies reactive with a protein which never was used in the immunisation procedure would be useful in obtaining antibodies against interacting proteins when only one of the proteins is available. Consequently anti-idiotypic antibodies against insulin would react with the insulin receptor. Preliminary observations in our laboratory suggest that this may indeed be the case.

This work was supported by grants from the Swedish Cancer Society and the Swedish Medical Research Council.

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Received 8 September; accepted 4 November 1977.

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Transient increase in muscarinic acetylcholine receptor and acetylcholine esterase in visual cortex on first exposure of dark-reared rats to light

AN organised sequence of cellular events, some transient, others more lasting, occurs in the visual system on first exposure of dark-reared rats to the light. Hitherto these changes have been biochemically characterised only in terms of rates of change of precursor uptake into protein fractions or *in vitro* assayed enzyme activities. We have shown that, in the visual cortex, the onset of visual experience is accompanied by a modulation of the total functional quantities of two classes of macromolecule with reasonably well understood functions in the cellular economy. One is the colchicine-binding microtubular protein, tubulin¹. We report here rapid transient changes in the amount of a protein known to be involved in synaptic functions, the muscarinic cholinergic receptor (mAChR), on the onset of light.

We showed previously that there is an elevation in the synthesis or turnover of several protein fractions in the visual cortex, as well as the lateral geniculate and retina^{2,3}. In the cortex, much of this increase is associated with a rapidly labelling and exported neuronal (glyco-)protein fraction⁴ whose transport is blocked by colchicine⁵. The microtubule system is apparently involved, since within 1 h of the onset of light, incorporation of ³H-lysine into a polymerisable tubulin fraction doubles⁶ and within 3 h there is a 23% elevation in the amount of colchicine-binding protein (largely tubulin) in the visual cortex¹. Over the same period, there is an increase in the activity of several acid hydrolases of up to 30%⁷.

All the changes listed above seem to be lasting, in that they persist for longer than 24 h and/or are present when dark-reared animals are compared to normally-reared littermates, with the exception of the elevation in colchicine-binding, which does not last beyond 24 h of light exposure. However, we have also found presumptive evidence for some apparently transient increases in the activity of the enzymes of acetylcholine metabolism, choline acetyltransferase (CAT) and acetylcholinesterase (AChE), in that while dark-reared animals did not differ in visual cortex enzyme activity from their normally-reared littermates, 3 h of light exposure of the dark-reared animals did produce a 30% elevation of activity of both enzymes⁷.

The transmitter(s) involved in the mammalian visual system are not known for certain, and the mere presence of relatively high concentrations of ACh and its enzymes is not, in itself, sufficient evidence for a transmitter role. However, the recent development of techniques for the detection and measurement of specific receptor binding proteins have shown the presence of significant quantities of a muscarinic cholinergic receptor at cortical synapses⁸. It has therefore become possible to examine the effects of dark rearing and visual experience on a molecule which may play a key part in the inter-neuronal transmission machinery.

Male Wistar rats were born and maintained in the dark until 50–55 d of age, at which time half of each litter was exposed, in individual cages, to normal laboratory illumination for 3 or 24 h (light exposed, L); the remainder were held in the dark, also in individual cages (dark controls, D). To avoid complications due to diurnal rhythmicity (N. Wood and S.P.R.R., in preparation) all exposures were carried out during the morning. In some experiments, a proportion of the littermates were removed from the dark at weaning (21 d) and subjected to a normal animal house

environment (12 h light, 12 h dark) until 50 d (normals, N). Experiments comparing L and D, D and N, and, in the AChE experiments, L, D and N groups, were made. Rearing conditions were exactly as described previously^{1,3}. Following treatments, L, D and N animals were killed, visual and motor cortex samples removed from each and homogenised in 0.05 M Na-K phosphate buffer, pH 7.4. Homogenates were either assayed directly or frozen until used. Preliminary experiments showed that freezing and thawing up to three times had no effect on the parameters to be measured.

Portions of each homogenate were assayed for: (1) muscarinic cholinergic receptor binding; (2) acetylcholinesterase; and (3) protein. Measurement of the muscarinic binding was based on the 3-quinuclidinylbenzilate (QNB) method of Yamamura and Snyder⁹ (see Table 1). Acetylcholinesterase (EC 3.1.1.7) was measured by the method of Ellman *et al.*¹⁰ and protein by that of Lowry *et al.*¹¹. Balanced groups were used in each experiment and batch variation in the mean QNB binding or AChE activity between experiments was eliminated by standardising results between batches. Statistical comparisons between groups were made using a two-tailed Student's *t*-test.

Figure 1 compares QNB binding in visual and motor cortex in 3- and 24-h light-exposed animals with that in their dark control littermates. At 3 h after the onset of light exposure, there was a 54% increase in QNB binding in the visual cortex of L compared with D animals ($t = 2.89$, $P < 0.01$). In the L animals, QNB binding in the visual cortex was 46% greater than in the motor cortex ($t = 2.33$, $P < 0.05$). There were no significant differences between binding in the visual and motor cortex of D animals, or between L and D animals in the motor cortex. However, the enhancement of QNB binding in the visual cortex of L animals was transient, as by 24 h binding activity had returned to the control level. A further indication that the effect was a transient response to the onset of light stimulation is given when QNB binding is compared in D and N animals (Table 1). There were no differences between animals in the two conditions, so light exposure cannot be necessary for the long-term normal development of cortical mAChR.

AChE activity was then measured in the same tissue samples. There were no differences in motor cortex levels

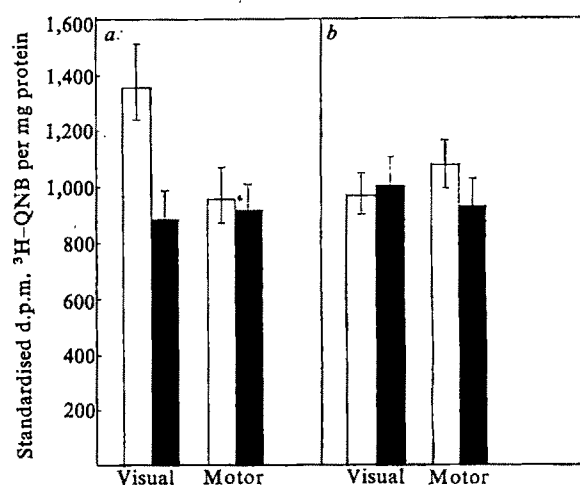


Fig. 1 ³H-QNB binding (black bars) was determined (open bars) in visual and motor cortices of dark-reared and light-exposed littermates aged 50–55 d as described in Table 1. Light exposure was for 3 h (a) or 24 h (b). Results are means of ≥ 16 animals in each case; error bars show s.e.m. Significance values are given in text. Results are expressed as standardised ³H-QNB radioactivity such that 1,000 d.p.m. \approx 1.0 pmol QNB bound per mg protein, but 3- and 24-h experiments are not directly comparable.

at either time, or between D and N animals in the visual cortex. There was a 24% elevation in visual cortex specific activity in L compared with D, and 18% in L compared with N, and both differences are significant (L versus D, $t = 3.94$, $P < 0.001$; L versus N, $t = 2.70$, $P < 0.02$). However, by 24 h the AChE activity in the L visual cortex had declined towards the controls and the difference is no longer significant ($t = 0.45$). The elevation of L over D at 3 h replicates our previous observation⁷ and the decline at 24 h confirms the prediction made in that paper.

These experiments, together with our earlier ones, demonstrate that the activities of the three components of the acetylcholine transmitter system in the visual cortex are responsive to the onset of visual input in dark-reared animals. CAT⁷, AChE and mAChR all show an elevation of specific activity of 24–54% within 3 h of the onset of light exposure; however, the response is transient, and by 24 h has disappeared.

These observations have two sets of implications. The first relates to understanding the mechanisms whereby experiential events (including learning) modulate neuronal connectivity. It is known that in the rodent, visual experience results in long-term changes in dendritic branching, spine formation and synaptic number and density^{12–14}. While enhanced protein synthesis and turnover of microtubular protein may underly these morphological changes^{2,4,6}, it seems that they do not involve a permanent increase in the activity of the ACh transmitter system per mg cortex protein. However, it is also well known that the fixation of experience in the brain is at least a two-stage process, the first being labile, the second more lasting, and that these stages can be uncoupled by the selective use of biochemical inhibitors (see, for example, ref. 15). Mobilisation of CAT–AChE–mAChR system could represent an aspect of synaptic potentiation associated with the labile phase of the fixation of experience. It is important to note that, unlike the enzyme activity measurements, those of mAChR involve a more direct assay of the number of

receptor molecules, or binding sites, present.

The second question raised by our observations relates to their molecular basis. Enhanced enzyme or binding activity could result from an activation or unmasking of pre-existing sites, or from a temporary increase in the *de novo* synthesis of the enzymes and receptors. To distinguish between these alternatives would provide evidence as to the nature of the control processes involved. Experiments to test this, and to clarify the subcellular locus of the enhanced QNB binding, are in progress.

We thank J. Mortimer for technical assistance, members of the Open University Brain Research Group for discussion and comments, T. Hedges for maintenance of the dark-reared rats and Dr G. B. Ansell of the University of Birmingham for advice on the assay procedure.

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Received 13 July; accepted 17 November 1977.

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Table 1 ³H-QNB binding in visual and motor cortex of dark-reared and normal littermates

Condition	Standardised ³ H-QNB binding (d.p.m./mg prot.) in:— visual cortex	motor cortex
Normals	983 ± 78 (16)	1,011 ± 107 (16)
Dark-reared	871 ± 125 (13)	923 ± 60 (12)

Aliquots of homogenate containing between 50 and 120 µg protein were incubated in a reaction mixture containing 52 pmol. ³H-QNB, specific radioactivity 8–8.9 Ci mmol⁻¹ (Radiochemical Centre, Amersham) in the presence or absence of 125 nM atropine and 0.05 M Na-K-phosphate buffer, pH 7.4, to a final volume of 2.0 ml. Incubations were at 25 °C for 60 min and each sample was assayed six times, three times with, and three times without, atropine present. After incubation, the reaction mixture was filtered under suction through 2.5 cm Whatman GF/B filter disks and washed rapidly three times with 5 ml ice-cold Na-K phosphate buffer. The disks were dried for 1 h at 45 °C and counted in a toluene-phosphor scintillant containing 0.2% v/v Triton X-100. Specific QNB binding (p mol per mg protein) was calculated from the mean difference in bound counts in the absence and presence of atropine. Preliminary experiments confirmed the applicability of the assay to unfractionated tissue homogenates. Binding in the presence of atropine was generally some 50–60% of that in its absence and increasing the atropine concentration did not further reduce it. No artefactual atropine-sensitive binding to the filters in the absence of tissue occurred. The variability within triplicate assays of the atropine-sensitive binding was generally within the range ±15% and assays that fell outside these limits were discarded or repeated. The assay was linear with increasing tissue concentration, and average binding, of 0.8–1.2 pmol QNB per mg tissue protein, was similar to that reported by Yamamura and Snyder⁹. Binding was determined in visual and motor cortices of dark- and normally-reared littermates aged 50–55 d. Values are ± s.e.m. with the number of animals tested shown in parentheses. There are no significant differences between any group. Standardisation is such that 1,000 d.p.m. ≈ 1.0 pmol QNB per mg protein.

Rapid accumulation of high molecular weight acetylcholinesterase in transected sciatic nerve

NERVOUS tissue and muscle in rat^{1–4} and chicken^{5,6} contain several molecular forms of acetylcholinesterase (EC 3.1.17, AChE), distinguishable by their sedimentation coefficient in sucrose gradient⁷. In rat, several peripheral nerves and non-innervated regions of skeletal muscle^{1,2} were shown to contain two molecular forms of AChE, with respective sedimentation coefficients of 4 and 10S; in addition to these two forms, a high molecular weight form with a sedimentation coefficient of 16S was found in the innervated regions of various skeletal muscles^{1,2,4}. After denervation, the 16S form, which we will call the H form, either disappeared from skeletal muscle² or was drastically reduced^{1,4}. In chicken, several nerves exhibited three molecular forms of AChE with sedimentation coefficients of 4, 6.5 and 11S, while various skeletal muscles contained an additional molecular form with a sedimentation coefficient of 19.5S (ref. 5). After denervation, the high molecular weight form 19.5S, which by analogy with the 16S of rat is referred to here as the H form, disappeared from crude extract of muscles⁵. As the H form was only found in tissues innervated by cholinergic nerve endings and disappeared or decreased drastically from denervated skeletal muscles, it has been suggested that this form of AChE is exclusively myogenic^{1,3} and may constitute the specific endplate enzyme^{1,2,4,5}. We report here that tiny amounts of the H form of AChE can be detected in crude extracts of intact sciatic nerves of rat and chicken, and that after transection of the sciatic nerve with the ensuing blockade of the axonal traffic, the H form increased rapidly at the site of injury.

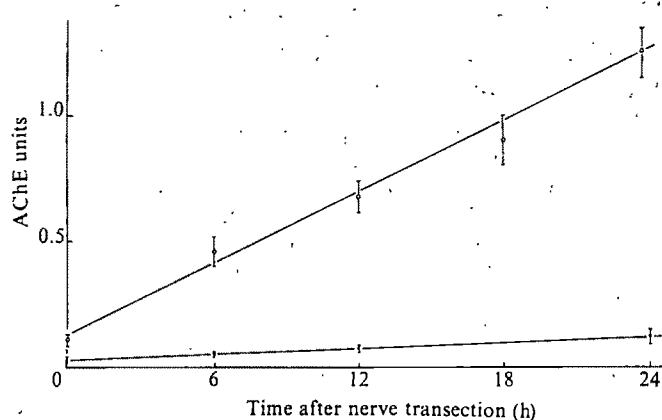


Fig. 1 AChE accumulation in transected sciatic nerve of rat (●) and chicken (○). Nerves were transected with scissors near the peroneal-tibial branching in rat and the ischiatic-femoral branching in chicken. At 6, 12, 18 and 24 h after transection, animals were killed and a 2-mm segment was cut off the tip of the proximal stump. The segment was homogenised in 1 M NaCl, 0.01 M Tris-HCl pH 7, 0.05 M MgCl₂ and 1% Triton X-100, and centrifuged at 20,000g for 20 min at 4 °C. The AChE was assayed in the supernatant at 20 °C, by the method of Ellman *et al.*¹² with acetylthiocholine as substrate and 10⁻⁴ M methopropazine as pseudocholinesterase inhibitor. One unit corresponds to hydrolysis of 73.5 nmol acetylthiocholine per min (ref. 13). Three animals per time-point were used.

Young Leghorn chicks (4 weeks old) and Wistar rats (2 months old) were anaesthetised with chloroform and one sciatic nerve was intersected by cutting out a 2-mm segment for use in measuring AChE concentrations in intact nerves (see legend to Fig. 1). At various intervals after transection (Fig. 1) the animals were killed and 2-mm segments cut off the end of the proximal stump. Nerve segments were then homogenised and the amount of AChE in the homogenate was determined (see legend to Fig. 1). Homogenates were then sedimented

through sucrose gradient to allow separation and characterisation of the different molecular forms of AChE (legend to Fig. 2).

In intact sciatic nerve of rat and chicken, AChE concentrations in the segment next to the site chosen for nerve transection were respectively 10 and 55 mU mm⁻¹ (for definition of unit see Fig. 1 legend). In both rat and chicken, separation procedure revealed the presence of four molecular forms of AChE (Fig. 2, 0 h). In rat sciatic nerve, we found a tiny amount (1.6%) of the H form (16.5S), one major form of 10S (M) which accounted for 63% of total AChE, and two low molecular weight forms (L) with respective sedimentation coefficients of 4S (L₁) and 6.5S (L₂), which accounted for 28 and 7.4% of total AChE. In chicken sciatic nerve, the H form (20S) only accounted for 1.3% of total AChE; the M form (73%) had a sedimentation coefficient of 11.5S; the two L forms had sedimentation coefficients of 5S (L₁) and 7S (L₂) respectively and accounted for 6 and 20% of total AChE. The presence of such small amounts of the H form in sciatic nerve of rat and chicken might explain why this form was not previously detected in nerve extracts^{1,2,5}. Detection of the H form, as well as of the 6.5S form in rat, which had also not been seen previously^{1,2}, was possible because of the higher resolution attained with the SW 41 Beckman rotor.

AChE activity in the 2-mm terminal segment from the proximal stump of the transected sciatic nerve, increased linearly during the 6th and 24th hours after transection (Fig. 1), at a rate of 4.2 mU h⁻¹ in rat and 47 mU h⁻¹ in chicken. To identify the molecular forms of AChE responsible for this rise in AChE activity, the crude extracts of the 2-mm segment were analysed by sedimentation in sucrose gradient. The sedimentation profile of AChE activity showed that only the activity of the two high molecular weight forms M and H increased with time, while that of the two low molecular weight forms L₁ and L₂ either remained unchanged or increased slightly (Fig. 2). The rise in the activity of the H form was very striking: 6 h after nerve transection, its concentration had increased 15-fold in rat and 20-fold in chicken (Fig. 3). For the M form, the rise was comparatively less pronounced, even though this form was responsible for most of the total increase in AChE activity,

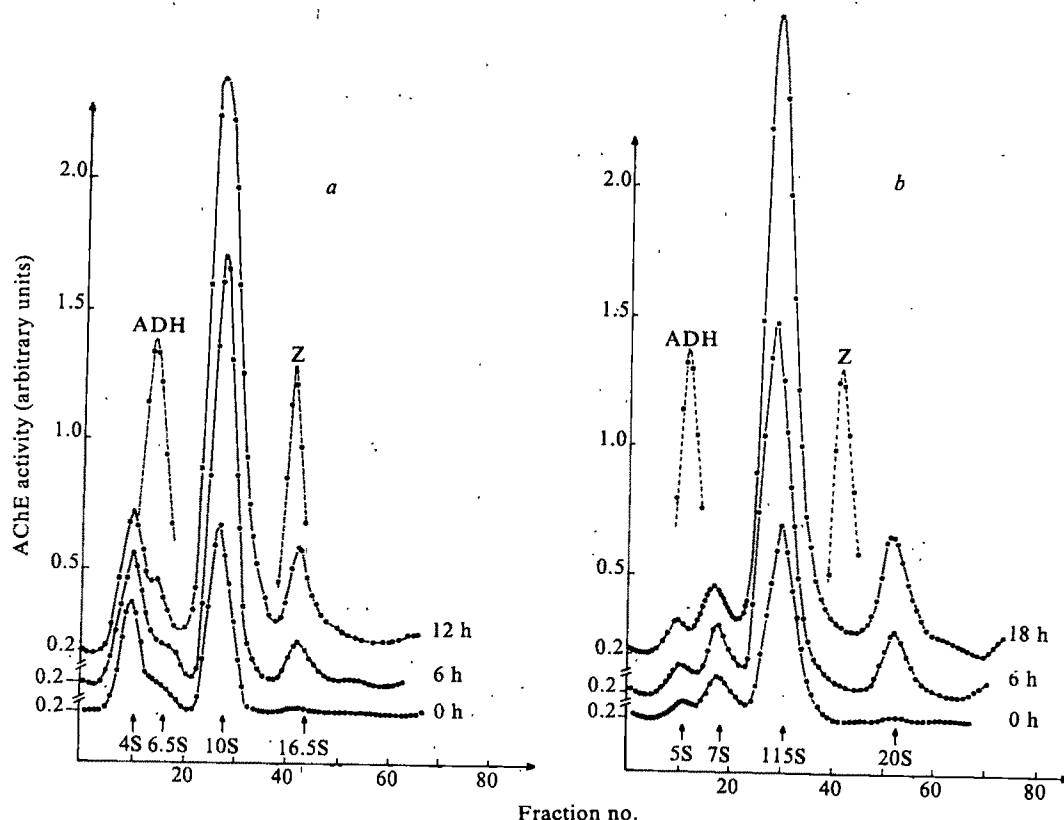


Fig. 2 AChE sedimentation profiles in intact (0 h) and transected sciatic nerves of rat (a) and chicken (b). The 20,000g supernatant (see Fig. 1) was mixed with two markers: ADH (alcohol dehydrogenase, 4.8 S, Boehringer) and Z (β-galactosidase, 16S, Boehringer), layered on a 5–20% sucrose gradient and centrifuged in a SW 41 rotor (Beckman). Approximately 70 fractions were collected. Each fraction was assayed for AChE, ADH and Z.

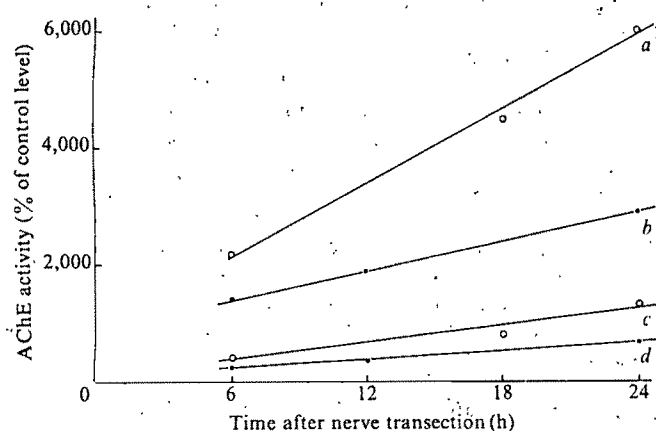


Fig. 3 Accumulation kinetics for the high molecular weight forms of AChE. The rise in the activity of each form at the 2-mm tip of the proximal stump is expressed as % of the same form's activity in the intact nerve (see Figs 1 and 2). *a*, Chicken, 20S; *b*, rat, 16S; *c*, chicken 11.5S; *d*, rat, 10S.

thus, 6 h after transection, M-form activity had doubled in the chicken and increased 1.5 times in the rat (Fig. 3).

In short, both rat and chicken sciatic nerves contain four molecular forms of AChE—two high molecular weight forms (H and M) and two low molecular weight forms (L_1 and L_2). After transection of the sciatic nerve, only the high molecular weight forms accumulated rapidly at the end of the proximal stump, whereas the low molecular weight forms either remained stationary or accumulated very slowly.

Previous work on dog⁸, cat⁹ and rats^{10,11} showed that after transection or ligation of the sciatic nerve, AChE accumulated at both sides of the lesion, at the speed of rapid axonal transport^{8,9,11}. In dog and cat, it was estimated that approximately 15% of the AChE present in the sciatic nerve was transported fast (10% outward and 5% inward), while the rest was either stationary or slow-moving^{8,9}. In rat, only 3% of the AChE in the sciatic nerve was transported fast¹¹.

Our results therefore indicate (1) that the H form of AChE is present in the intact nerve of rat and chicken and (2) that this and the M form (the other high molecular weight form) move with the rapid phase of axonal transport, whereas the low AChE molecular weight forms L_1 and L_2 , also present in the intact nerve of rat and chicken, either remain stationary or move with the slow phase of axonal transport.

This work was partly supported by INSERM grant CR No. 77.4.180.6C.

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Cholinesterase is associated with the basal lamina at the neuromuscular junction

THE cholinesterase (ChE) of skeletal muscle is concentrated at the neuromuscular junction, where it hydrolyses acetylcholine released from the nerve terminal^{1–3}. In frog muscle treated with a histochemical stain for ChE, enzyme activity is demonstrable at all terminal branches of the neuromuscular junction (Fig. 1*a*), with the reaction product occupying the synaptic cleft between nerve and muscle (Fig. 1*d*)^{1,6}. A particulate enzyme, ChE, was assumed to be integral to the postsynaptic plasma membrane² until Hall and Kelly⁷ showed that mild protease treatment of intact muscles released active ChE into the medium without apparent damage to the plasma membrane. One possibility raised by this result is that ChE is associated with the basal lamina (BL; sometimes called basement membrane) that runs through the synaptic cleft⁸. This idea was supported by the finding that a subunit of ChE from electric organs of fishes and rays has several properties in common with collagen^{9–11}, a protein known to be a principal component of BLs¹². Here, we present direct evidence that junctional ChE is associated with the BL of the synaptic cleft in skeletal muscle.

Our approach was to remove the cellular components of the neuromuscular junction *in vivo* (nerve terminal, Schwann cell, and muscle cell; Fig. 1*d*) leaving behind the BL; we then assayed ChE histochemically. The nerve to the cutaneous pectoris muscle of the frog was cut, causing degeneration of the nerve terminals¹³. A long portion of nerve was removed to maintain denervation, inducing Schwann cell processes to retract from synaptic areas¹³. Muscles were injured mechanically (see Fig. 1 legend and ref. 14), causing disruption of muscle fibres; the damaged cells decomposed, leaving behind the sheaths of BL that surrounded them^{14,15}. Frogs were X-irradiated to prevent regeneration of new muscle within the BL sheaths (ref. 16 and J.R.S., L.M.M. & U.J.M., in preparation). Cellular debris and membrane fragments within the sheaths were phagocytosed^{14,15} and electron microscopy showed that nearly all ($98.3 \pm 0.4\%$; mean \pm s.e.m. of samples from 41 sheaths) of the surface area of the BL was clean by two weeks after the muscle was damaged. Portions of such sheaths are shown in Fig. 1*c*.

When we stained such preparations for ChE^{8,13,17} and examined them under the light microscope, bands of reaction product (Fig. 1*b*) corresponding in size, shape and arrangement to the ChE-outlined terminal arborisations of normal muscle (Fig. 1*a*) were revealed. In addition, the bands had a periodic substructure corresponding to that of the junctional folds (Fig. 1*d*), which are arranged at intervals of approximately $1 \mu\text{m}$ along the endplate⁶. (The periodic variation in staining intensity is particularly prominent after the overlying nerve terminal and Schwann cell processes are removed by denervation, whether the muscle is damaged or not¹³.) Thus, staining for ChE showed that the enzyme was concentrated at junctional sites on the sheaths.

Electron microscopy of the sheaths confirmed that ChE was associated with BL. Reaction product was concentrated in and near the BLs that had occupied the synaptic cleft and projected into junctional folds (Fig. 1*e*). Because reaction product might have obscured remnants of the muscle at junctional sites, we examined preparations not stained for ChE, using the BLs from junctional folds and Schwann cells (Fig. 1*e*) to identify junctional sites on the sheaths¹⁴. Less than 2% of the junctional BL ($1.8 \pm 0.5\%$; $n = 28$) was apposed by fragments of plasma membrane and this is far too little to account for the even pattern of

ChE staining seen light and electron microscopically.

Light microscopic observations provided additional information about the ChE associated with BL. ChE-stained bands on the BL sheaths were fainter than those on normal fibres; it is known that levels of junctional ChE decrease following denervation^{1,2,13,18}. ChE-stained bands on the sheaths were similar in intensity to those on denervated,

but undamaged, fibres in the same preparations; thus, a major fraction of the ChE that survived denervation remained with the BL after the muscle was removed. The intensity of staining was reduced by BW 284C51, a specific inhibitor of 'true' acetylcholinesterase (EC 3.1.1.7) (ref. 2), demonstrating that some, if not all, of the ChE in the BL is acetylcholinesterase.

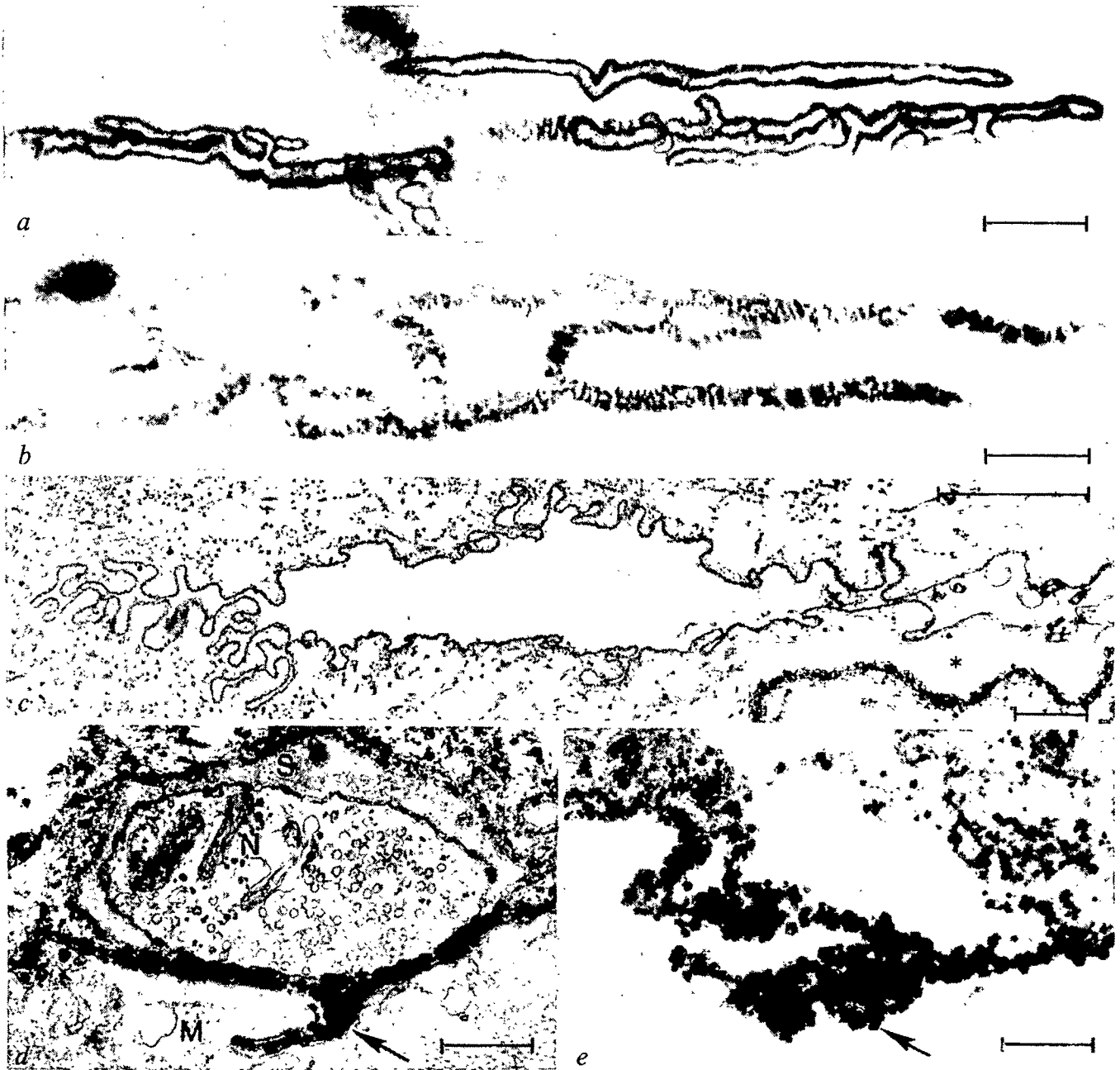


Fig. 1 *a, b*, Light micrographs of ChE-stained endplates (sites of neuromuscular junctions) on a normal muscle fibre (*a*) and on a BL sheath that survived denervation and muscle damage (*b*). Bars represent 20 μm . *c*, Low-power electron micrograph of a cross section through a BL sheath. Collagen fibrils lie outside the BL and the inside, once occupied by the muscle fibre, is now nearly empty. Bar represents 2 μm . Inset: high-power micrograph of a portion of a BL sheath demonstrates the absence of muscle cell plasma membrane which is normally situated 10–20 nm from the BL. Asterisk is in the lumen of sheath. Bar represents 0.2 μm . *d, e*, Electron micrographs of ChE-stained junctional sites from preparations similar to those shown in *a* and *b*, respectively. Muscle cell (M), Schwann cell process (S), and nerve terminal (N), all present at the normal neuromuscular junction (*d*), disappear following denervation and muscle damage (*e*), leaving behind the BL that surrounded muscle and Schwann cell and projected into junctional folds (arrows). The muscle in *d* was stained lightly for ChE, to avoid obscuring cellular elements. Bars represent 0.5 μm . All experiments were carried out on the cutaneous pectoris muscles of 5-cm male *Rana pipiens*. To prepare BL sheaths, frogs were submitted to the following regimen: On day 1, the nerve to the cutaneous pectoris was cut within 1 mm of the muscle border, and a 2-cm segment of nerve was evulsed. On day 12, pieces were cut from each end of the muscle, leaving behind a central row of 1–1.5-mm long muscle fibre segments, many of which bore endplate sites (details in ref. 14). On day 15, frogs were X-irradiated (250 kV; 15 mA; added filter of 2 mm Al; 65 cm from source to centre of animal; 100 rad min⁻¹; total dose, 1,600 rad). Lead shielding placed over the frog restricted radiation to the thorax. On day 26, animals were killed and muscles were anaesthetised with MS 222. Normal (*a, d*) and damaged (*b, c, e*) muscles were prepared for microscopy as described previously^{6,13}. Ruthenium red was used to stain BL for electron microscopy¹⁴. The histochemical procedure of Karnovsky¹⁷ was used to demonstrate ChE activity^{6,13}. Results similar to those illustrated here were also obtained with muscles that were denervated and damaged on day 1, irradiated on day 3, and fixed 3–5 weeks later.

Thus, at least some of the ChE at the frog neuromuscular junction is very tightly bound to (that is, contained in or connected to) the basal lamina of the synaptic cleft. To our knowledge, this is the first case in which an enzyme has been shown to be associated with the basal lamina of any cell.

We thank Mary Hogan and Linda Yu for assistance, and Dr Henry Kohn for help with X-irradiation. This work was supported by the US Public Health Service and by a Research Fellowship (to L.M.M.) of the Muscular Dystrophy Association.

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Received 19 July; accepted 9 November 1977.

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Effect of calcium ionophore Br-X537A on renin synthesis and release in *Amphiuma means* kidney culture

CALCIUM ions play an important part in stimulus-secretion coupling¹, and in particular have been implicated in the control of renin release from kidneys *in vitro*². The calcium ionophores A23187 and X537A and its derivatives have been used in several studies of the effect of mobilisation and movement of calcium and other divalent ions on biological systems, including mast cells, pancreatic B cells and the neurohypophysis³⁻⁸. X537A and Br-X537A are of interest because they release calcium preferentially from intracellular sites irrespective of the extracellular calcium concentration^{8,9}. In investigating calcium involvement in secretion and the action of ionophores, emphasis has been placed on *in vitro* studies, where the experimental conditions can be closely controlled. We report here the results of experiments on the effect of Br-X537A on the renin levels in *Amphiuma means* kidney in organ culture. This system has the advantages of an isolated tissue, which in addition retains its *in vivo* characteristics for extended periods. We show that Br-X537A has dose-dependent effects on both the synthesis and secretion of renin. These effects apparently represent two quite separate sites of action of the ionophore.

Control of renin secretion is effected through humoral agents, renal sympathetic nerve stimulation, and the intra-renal receptors sensitive to pressure or to ion concentrations¹⁰. Renin release by the kidneys is of fundamental importance in the maintenance of blood pressure and sodium homeostasis. The interpretation of *in vivo* studies of drug action on renin secretion is complicated by the interplay of the control mechanisms¹⁰⁻¹³. To distinguish between direct and indirect actions of drugs on renin release, isolated perfused kidney¹⁴ and kidney slices^{2,15} have been used *in vitro*. These methods avoid the complications

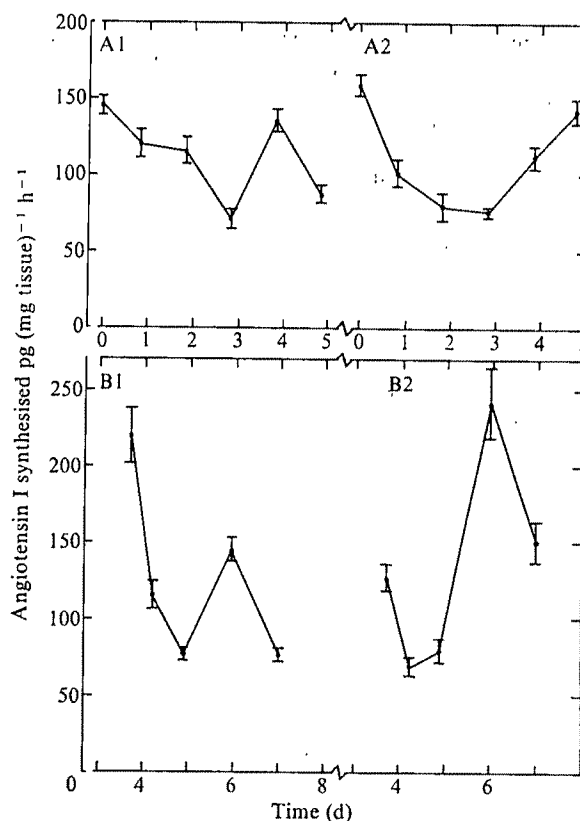


Fig. 1 The renin activity of *A. means* kidney tissue in culture over several days after initiation of culture. Kidneys from two freshly killed animals were immediately removed to culture medium and cut into pieces of average weight 1.5 mg. The pieces were cultured (about 25 mg per 2 ml of medium) in sterile polystyrene universal containers on a roller culture apparatus at 25 °C. Basic culture method was as described by Monnickendam and Balls²⁰. The medium was 50% Eagle's minimum essential medium with 10% foetal calf serum buffered with HEPES (15 mM). Streptomycin sulphate (100 µg ml⁻¹) and benzyl penicillin (100 IU ml⁻¹) were added. Renin activities were measured by radioimmunoassay of angiotensin I generated from exogenous renin substrate, using a method based on that of Cohen *et al.*²¹ and Haber *et al.*²². The renin activities are expressed as the amount of angiotensin I generated per mg of tissue (wet weight). The bars represent s.e.m., and the number of cultures for each point was either 7 or 8, except for day 0 (fresh tissue) where renin activities were measured in tissue equivalent to three cultures. Each trace shows the renin activity in tissue from a single kidney: A1 and A2 referring to the two kidneys from one animal (measurements made from day 0 to day 5); B1 and B2 to kidneys from the second animal (day 3 to day 7).

of haemodynamic and humoral factors. However, it is known that tissue slices from adult mammalian kidneys lose their normal structure and function in quite short periods ranging from 20 s to 3 d, depending on the experimental conditions¹⁶⁻¹⁹.

By contrast, many amphibian tissues in organ culture maintain their structure and biochemical functions for periods of up to 5 weeks²⁰. We have shown that *A. means* kidney in culture maintains its renin content for several days (see Fig. 1). This system is therefore useful for studying the long- and short-term effects of drugs without the complications of changes in haemodynamic and sympathetic nervous activity.

As shown in Fig. 1, after an initial drop in tissue renin activity at the start of the culture period, the renin activity fluctuates with time. Cultures from one kidney behave quantitatively in the same way. Tissue from the second kidney of the same animal may show quite different fluctuations. In studying the effect of the ionophore we have, therefore, related renin activities in the tissue and culture medium at given times to those of controls using tissue from the same kidney.

Table 1 Effect of Br-X537A and puromycin on renin activities in *A. means* kidney and culture media

Treatment	Tissue	Renin activity (pg angiotensin I mg tissue ⁻¹ h ⁻¹) Medium	Total
None (control)	160.7 ± 3.0 (9)	9.7 ± 0.9 (9)	170.4 ± 3.4
Br-X537A 2.12 µg ml ⁻¹	357.4 ± 8.2 (8)*	16.9 ± 2.7 (8)*	374.3 ± 9.3*
None (control)	83.9 ± 2.4 (6)	15.0 ± 1.8 (6)	98.9 ± 3.3
Br-X537A 11.5 µg ml ⁻¹	157.0 ± 4.5 (6)*	40.0 ± 1.9 (6)*	197.1 ± 3.3*
Puromycin 0.25 mM	82.8 ± 2.4 (7)	11.6 ± 1.2 (7)	94.4 ± 2.8
None (control)	154.4 ± 12.7 (5)	15.3 ± 1.6 (5)	169.8 ± 14.2
Br-X537A 20.5 µg	129.8 ± 3.8 (5)	34.4 ± 5.0 (5)†	164.2 ± 6.2
None (control)	184.3 ± 2.5 (6)	19.1 ± 1.2 (6)	203.4 ± 2.9
Puromycin 0.25 mM + Br-X537A 11.5 µg ml ⁻¹	160.0 ± 2.8 (6)*	22.0 ± 3.0 (6)	181.7 ± 5.6†

Results are expressed as mean ± s.e.m. with number of cultures in parenthesis. The culture medium and conditions, and method of renin assay were as described in Fig. 1 and the text.

* $P < 0.001$, in comparison between treated and control values in the same experiment.

† $P < 0.01$.

For drug treatments fresh kidney tissue was cultured at 25 °C for 20–24 h. The medium was then changed to either fresh medium (controls) or to fresh medium containing the drugs. After treatment for 24 h the kidney pieces were removed, rapidly blotted, weighed, frozen and kept at –20 °C until assayed for renin activity. Media containing the ionophore were prepared from solutions of the drug in dimethyl sulphoxide. In control cultures dimethyl sulphoxide was added to give the same concentration as in the treated cultures. The concentration of the solvent never exceeded 0.5% v/v. Lactate dehydrogenase (LDH) activity in the medium was measured to check for nonspecific leakage of cell contents. The amount of LDH in the medium was less than 0.5% of that measured in the tissue.

The effects of Br-X537A and puromycin on renin activity are shown in Table 1 and Fig. 2. Over 24 h puromycin alone has no effect. The ionophore gives two dose-dependent effects on renin levels. The first effect is on renin synthesis. There is a stimulation of total (tissue + medium) renin

activity at low ionophore concentrations, and this effect is progressively inhibited as the ionophore concentration is increased. That this is due to enhanced renin synthesis and not to some other action of the ionophore is indicated by the observation that the presence of both puromycin and ionophore (11.5 µg ml⁻¹) together causes only a small decrease in the total renin activity. The second dose-dependent effect is on renin release. In order to distinguish the effect on renin release as distinct from synthesis, we can express the medium renin activity as a fraction of the total renin activity present, f . Then to take into account the variability between different kidneys, f is normalised to the control value (see Fig. 2 insert). Then it is apparent, that puromycin has no significant effect on renin release and, as has been observed by other workers using the neurohypophysis⁸, the amount of hormone released increases with ionophore concentration. However, the validity of interpreting the results quantitatively this way depends on all the tissue renin being equally available for release.

The secretagogue^{4,8}, pseudofertilisation²³, and cardiovascular⁶ actions of X537A and Br-X537A are usually interpreted as being mediated by mobilisation of Ca²⁺ ions and/or catecholamines. We have been unable to show a significant effect of isoprenaline or adrenaline on renin levels in *A. means* kidney *in vitro* over the time scale of the present experiments (data not shown); this suggests that intracellular Ca²⁺ activities are critical in determining renin synthesis, in a manner reminiscent of steroidogenesis in the adrenal cortex²⁴, and synthesis of hormones in the pituitary²⁵. We have been able to demonstrate the two separate actions of Br-X537A on renin levels (synthesis and secretion), because we have used an *in vitro* kidney system that retains its renin for extended periods.

This work was supported by a grant from the East Anglian Hospital Board. We thank Dr I. Atwater and Professor E. Rojas for useful discussions, Dr M. Balls for help in initiating our work on kidney organ culture, and Hoffman-LaRoche for Br-X537A.

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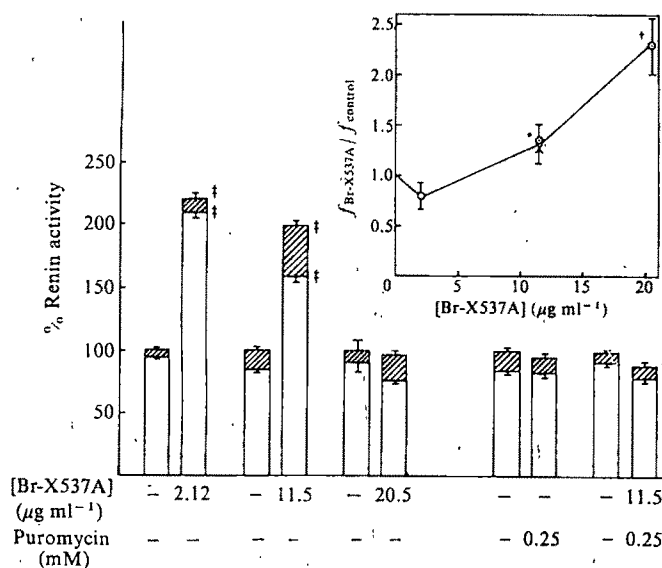
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Fig. 2 To show the different dose dependences of renin synthesis and release, renin activities are shown as a % of the total control values. Open bars represent tissue activity and cross-hatched areas represent medium renin activity. Error bars represent s.e.m. The insert shows $f_{\text{Br-X537A}}/f_{\text{control}}$, (fraction of total renin in medium in the presence of ionophore/fraction of total renin in medium in untreated control cultures) as a function of ionophore concentration. ×, Puromycin present. *, $P < 0.05$, †, $P < 0.01$, ‡ $P < 0.001$, where comparisons are made between cultures with and without drugs. In the insert, the P values refer to comparing $f_{\text{Br-X537A}}$ with f_{control} .



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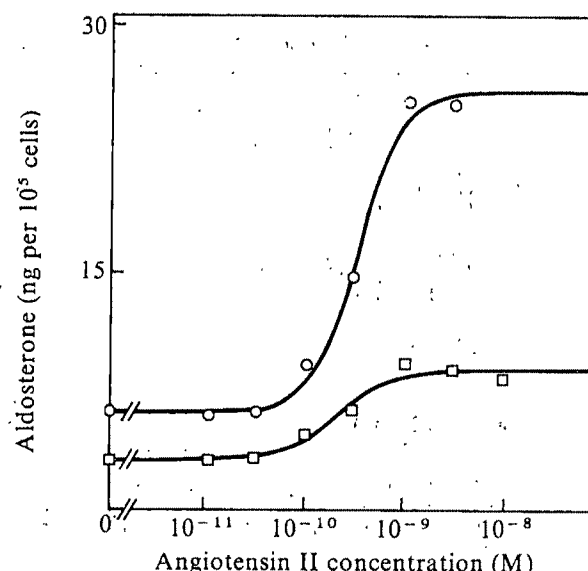


Fig. 1 Stimulation of aldosterone production by angiotensin II in glomerulosa cells from control rats (\square) and rats receiving angiotensin II infusion for 36 h (\circ). Isolated glomerulosa cells were prepared from adrenal capsules of 50 animals in each of the control and angiotensin-infused groups.

Angiotensin II regulates its receptor sites in the adrenal glomerulosa zone

DURING sodium deficiency, adrenocortical sensitivity to angiotensin II is increased in several species¹⁻³. Following acute sodium restriction in the rat, adrenal receptor sites for angiotensin II show increased concentration and binding affinity, and aldosterone responses to angiotensin II are increased both *in vivo* and *in vitro*⁴. Since circulating renin and angiotensin II levels are elevated during sodium deficiency^{2,3}, these changes in the adrenal gland could be mediated by the trophic action of angiotensin II on the zona glomerulosa. Increased levels of catecholamines and trophic peptide hormones, such as luteinising hormone, have been shown to cause receptor loss and desensitisation of specific responses in their respective target cells⁵⁻⁷. It is possible, however, that physiological elevations of blood angiotensin II concentration could exert the opposite effect in the adrenal zona glomerulosa, leading to enhanced receptor function and contributing to the increased adrenal sensitivity during sodium restriction. To determine whether angiotensin II modulates its own receptors as well as the aldosterone secretory response, we examined angiotensin II receptors and aldosterone production in the zona glomerulosa of rats following elevation of circulating angiotensin II levels. These studies have provided evidence that angiotensin II can regulate its own receptor sites as well as steroidogenic responsiveness in the adrenal glomerulosa cell.

Isolated zona glomerulosa cells and membrane-rich particles were prepared from adrenal glands of adult rats as described previously⁸. Circulating angiotensin II levels were increased by implanting Alzet osmotic minipumps (Alza Corporation, Palo Alto) intraperitoneally for periods of 36 h-6 d, to give sustained release of synthetic [Asp¹, Ile³]angiotensin II (Beckman Instruments, Inc. Palo Alto), at a rate of 50 ng min⁻¹. The control rats were implanted similarly with empty plastic tubing for equivalent periods. Assay of adrenal receptors by binding of ¹²⁵I-angiotensin II, and measurement of aldosterone in plasma and incubation media, were carried out as described previously^{8,9}.

Plasma aldosterone concentrations were markedly increased from 9.8 ± 2.5 ($n = 12$) to 26.9 ± 5.8 ng per 100 ml ($n = 13$), following infusion of angiotensin II for 36 h-6 d ($P < 0.001$). Angiotensin II infusion was also followed by enhancement of basal and angiotensin-stimulated aldosterone production in isolated glomerulosa cells (Fig. 1). After 36 h of infusion, the maximum aldosterone response *in vitro* was increased from 9.2 ± 0.4 to 26.0 ± 1.3 ng ml⁻¹

($P < 0.001$). The increased maximum response to angiotensin II *in vitro* was not accompanied by a change in sensitivity to the peptide, as reflected in the angiotensin concentration required to achieve a half-maximum aldosterone response. This rapid and marked change in adrenal responsiveness was accompanied by commensurate changes in the concentration of angiotensin II receptors in the adrenal glomerulosa cell (Fig. 2). A significant increase in the number of angiotensin II receptors was observed after 36 h of angiotensin II infusion, with no change in the association constant of the angiotensin II receptor. Similar increases in the angiotensin II binding capacity (that is, in receptor sites) were observed in adrenal subcellular particles after 36 h (Fig. 3). Such changes were observed for up to 6 d of infusion, whereas receptor affinity was not significantly altered (Table 1).

The rate of angiotensin II infusion used in these studies (50 ng min⁻¹) was accompanied by a slight increase in sodium excretion by the experimental animals. This sodium loss may possibly contribute to changes in angiotensin II receptors and adrenal responses, albeit by an as yet undefined mechanism. The same effects were also observed following infusion of angiotensin II at a rate (20 ng min⁻¹) that had no detectable influence on sodium excretion, however, thus emphasising the ability of the octapeptide to act directly on adrenal receptors and steroidogenic responses.

This study has demonstrated that infusion of angiotensin II causes rapid and significant increases in both angiotensin II receptors and angiotensin-induced responses in the adrenal glomerulosa cells. In contrast, exposure of other target tissues to high hormone concentrations frequently results in reduction of receptor sites and biological effects of the regulatory hormone. Such 'desensitisation' phenomena have been found for luteinising hormone and catecholamines⁵⁻⁷. Other peptide hormones such as insulin, growth hormone, and thyrotropin-releasing hormone decrease their own receptors¹⁰⁻¹², whereas high circulating prolactin concentrations produced by oestrogen treatment are believed to increase the hepatic prolactin receptor¹³. In tissues in which receptors are decreased by elevations of the homologous hormone, an increase in hormone receptors would be expected when the circulating hormone is decreased. Accordingly, both thyrocalcitonin receptors and

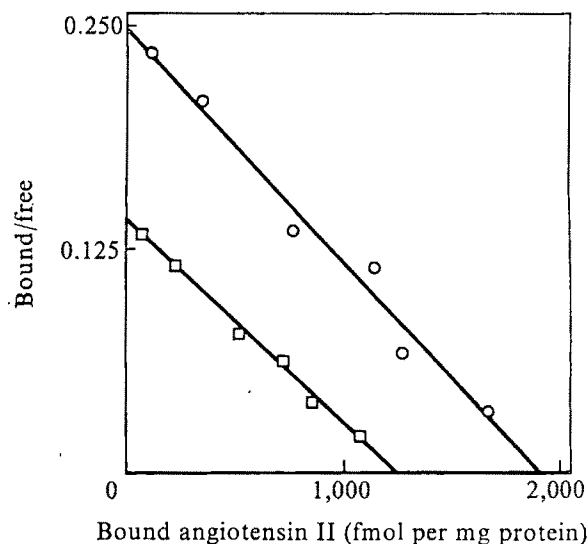
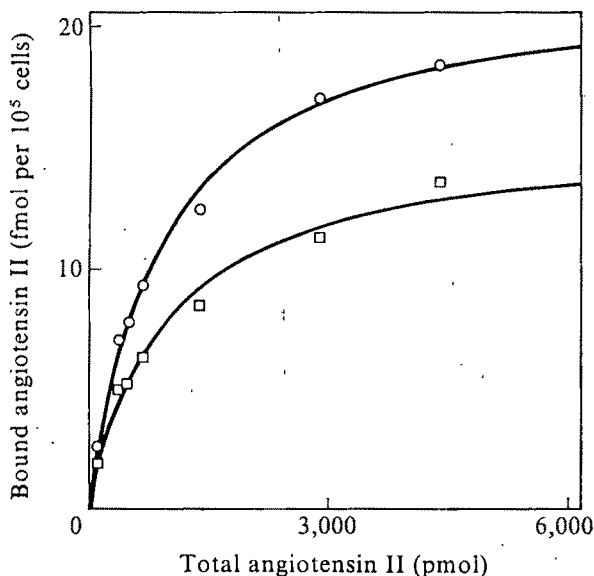
Table 1 Concentration and binding affinity of angiotensin II receptors in adrenal of control and angiotensin II-infused rats

Duration of infusion (d)	Control		After angiotensin II infusion	
	Concentration (fmol mg ⁻¹)	K _a (× 10 ⁹ M ⁻¹)	Concentration (fmol mg ⁻¹)	K _a (× 10 ⁹ M ⁻¹)
1.5	1,188 ± 40	0.53 ± 0.04	1,766 ± 109	0.56 ± 0.09
3.5	1,697 ± 120	0.84 ± 0.13	2,463 ± 263	0.69 ± 0.12
3.5	1,288 ± 39	1.79 ± 0.03	1,629 ± 74	0.88 ± 0.05
6.5	1,160 ± 52	0.76 ± 0.04	1,911 ± 123	0.61 ± 0.03

Angiotensin II receptor concentrations for all experiments significantly increased ($P < 0.02$) from $1,333 \pm 124$ to $1,943 \pm 183$ (mean ± s.e.) following angiotensin II infusion. Each of the four experiments was performed on adrenal particles prepared from 15 rats in the control and angiotensin-infused groups.

uterine angiotensin II receptors are increased by the absence of the regulatory hormone^{14,15}. The adrenal angiotensin II receptor, however, seems to be regulated in the opposite manner. Thus, angiotensin II receptors in the rat adrenal are reduced after nephrectomy¹⁶ and aldosterone responses of the human zona glomerulosa are reduced following the removal of endogenous angiotensin II by nephrectomy and restored after renal allografts¹⁷. Conversely, sodium restriction, which is associated with increased circulating angiotensin II levels, increases angiotensin II binding sites and the sensitivity and magnitude of the aldosterone response^{2-14,18}.

Administration of pharmacological doses of angiotensin II has been shown to produce a decrease in adrenal receptor sites in nephrectomised rats¹⁸. Such depletion of peptide receptors probably occurs in all target cells after excessive degrees of receptor occupancy, and may bear little relevance to the physiological modulation of target cell functions by normal variations in circulating hormone concentration. In contrast, the administration of nanogram quantities of angiotensin II in the present study was accompanied by a rise in specific adrenal receptors, in keeping with the enhanced aldosterone production seen *in vitro* and *in vivo* after angiotensin infusion. It should be noted that the level of plasma aldosterone produced by angiotensin II infusion was less than that observed during similar periods of sodium deficiency, namely 69.2 ± 7.2 ng per 100 ml after 4 d. This

Fig. 2 Saturation curves of angiotensin II binding data derived with adrenal glomerulosa cells from control rats (□) and rats receiving angiotensin II infusion from 36 h (○). Binding analysis was on the cell preparations used for the aldosterone dose-response studies shown in Fig. 1.**Fig. 3** Scatchard plots of angiotensin II binding in adrenal glomerulosa 30,000g particulate fraction from control rats (□) and rats receiving angiotensin II infusion for 36 h (○). Each set of binding data was derived from adrenal particles prepared from 15 control and 15 angiotensin-infused animals.

difference could reflect the need for a pulsatile or episodic pattern of increased angiotensin II secretion to produce the maximum trophic action on zona glomerulosa cells. The presence of diurnal variation and episodic secretion of renin is well established in man, and pulsatile change may form an important component of the modulating effect of angiotensin II on adrenal glomerulosa function and aldosterone secretion.

We suggest that the direct regulatory effect of angiotensin II on the zona glomerulosa is a major factor in the increased responsiveness of the adrenal cortex during sodium restriction. This view is consistent with earlier studies which showed the administration of renin increases adrenocortical sensitivity to angiotensin II in the dog¹⁹, and with the recent finding that prolonged infusion of angiotensin II in man similarly increases the sensitivity of the adrenal cortex²⁰. Such changes are also accompanied by an increase in ACTH-induced aldosterone responses, indicating a generalised enhancement of the aldosterone biosynthetic pathway^{19,21} that is consistent with a trophic action of angiotensin II on the adrenal gland. In conjunction with these observations, our findings of angiotensin II receptor induction and correspondingly increased steroid responses provide direct evidence that angiotensin II exerts a trophic action on cells of the zona glomerulosa, in addition to its acute regulatory effects on aldosterone secretion.

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Evidence for a long Leu-enkephalin striopallidal pathway in rat brain

THE pentapeptides leucine (Leu-) enkephalin and methionine (Met-) enkephalin were isolated from brain and shown to be endogenous opiates¹. These peptides bind to the brain opiate receptor², and their biological actions are blocked by the morphine antagonist naloxone³. The availability of synthetic enkephalins has led to the development of radioimmunoassays (refs 2, 4, and R. Miller, personal communication) and immunohistochemical techniques. Preliminary immunohistochemical studies^{5,6} have shown the distribution of enkephalin-containing fibres to coincide with the regional distribution of opiate receptors as determined by biochemical techniques and autoradiography⁶⁻⁸. The highest density of enkephalin-positive fibres^{8,9} was located in the globus pallidus. We have now confirmed the presence of enkephalin-immunoreactive fibres in the globus pallidus, and using specific lesions have shown this immunoreactivity to be abolished by destruction of the neural connections between the globus pallidus and the caudoputamen.

The visualisation of the Leu-enkephalin-immunoreactive fibres in the brain tissue was carried out using the indirect fluorescent immunohistochemical techniques first described by Coons and Kaplan⁹ with a Leu-enkephalin antiserum from rabbit and anti-rabbit IgG fluorescein conjugated immunoglobulin (Wellcome). Rat brains were fixed by intracardiac perfusion with 4% cold paraformaldehyde in 0.1 M buffer phosphate and processed as described by Hökfelt *et al.*¹⁰. Cryostat sections 10-µm thick were used for immunocytochemistry. The exact location and extent of each lesion was determined from Nissl-stained sections. Leu-enkephalin antiserum was raised in the rabbit by Dr R. Miller of Burroughs Wellcome. The Leu-enkephalin antiserum did not cross-react with β -lipotropin or adrenocorticotrophic hormone (ACTH), and the cross-reactivity with Met-enkephalin was 1% and with β -endorphin < 0.01%. The specific immunofluorescence in the globus pallidus and other regions was not observed if the Leu-enkephalin antiserum was previously absorbed with Leu-enkephalin (200 µg ml⁻¹). Light microscopy at high magnification showed the immunoreactivity in the globus pallidus to form a dense mesh of intensely fluorescent elements. The lack of immunoreactive elements in the caudoputamen and the abundance in the globus pallidus causes a sharp delineation of the border between caudoputamen and the globus pallidus.

Isolation of the globus pallidus from the neighbouring caudoputamen by a knife-cut lesion within the caudoputamen (Fig. 1, lesion 1) caused an almost complete depletion of Leu-enkephalin immunofluorescence from the ipsilateral globus pallidus (Fig. 2a, b). In 10 animals, the magnitude of this depletion was related to the extent of the lesion in the caudoputamen determined from alternate Nissl-stained sections. Knife cuts limited to the rostral pole of the caudoputamen (Fig. 1, lesion 2) produced a depletion of enkephalin immunoreactivity restricted to the medial and rostral portions of the globus pallidus. This observation suggests a possible topographical arrangement of the enkephalin-containing fibres in the caudoputamen-globus pallidus pathway.

The view that the Leu-enkephalin might be present in the processes of striatal neurones was reinforced by the appearance

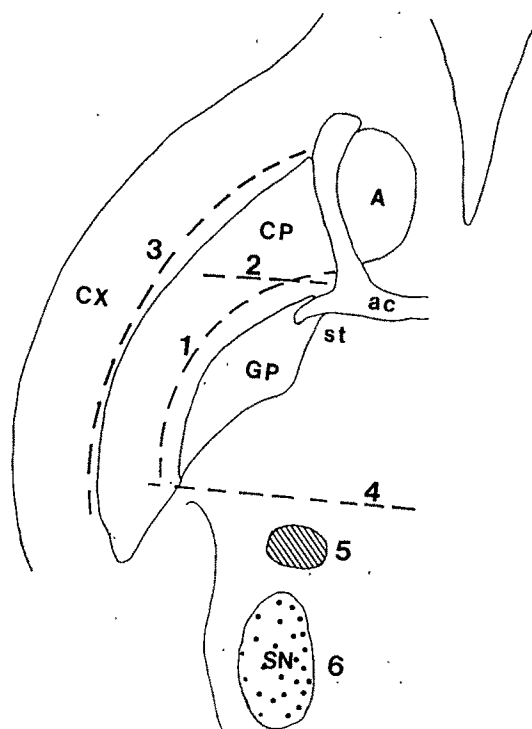


Fig. 1 Schematic representation of a horizontal section of the rat brain. CX, cortex; CP, caudoputamen; GP, globus pallidus; A, nucleus accumbens; ac, anterior commissure; st, nucleus interstitialis of the stria terminalis; SN, substantia nigra. 1, Knife cut isolating the caudoputamen from globus pallidus; 2, knife cut in the rostral pole of the caudoputamen; 3, cerebral cortex knife undercut; 4, brain hemisection; 5, electrolytic lesion of the medial forebrain bundle; 6, intranigral injection of 6-hydroxydopamine. Lesions 1-4 were performed with a stereotactically-guided microknife. Survival time for all procedures was 5-8 d.

of bundles of fluorescent beaded fibres on the striatal side of the knife cuts (Fig. 3). These fluorescent fibres were only seen on the lesioned side, running radially through the striatum towards the globus pallidus. When consecutive sections were incubated with non-immune serum or anti-enkephalin serum pre-absorbed with Leu-enkephalin (200 µg ml⁻¹) the accumulation of fluorescent material proximal to the lesion was not observed. The build-up of enkephalin immunofluorescence observed here is similar to that seen with substance P after dorsal root lesions¹⁰ or knife cuts in the central nervous system¹¹.

Stereotaxic undercutting of the cerebral cortex with a microknife (Fig. 1, lesion 3) did not produce any noticeable change in the intensity or pattern of the pallidal immunofluorescence compared with the contralateral untreated side. Stereotaxic injections of 6-hydroxydopamine (8 µg in 2 µl given over 4 min) into the substantia nigra which in parallel experiments resulted in a 60-70% fall in striatal dopamine content, electrolytic lesions of the medial forebrain bundle (2 mA, 20 s), and stereotaxic knife-cut hemisections at the middle-hypothalamic level, all failed to change the pattern of Leu-enkephalin immunoreactivity in the globus pallidus (Fig. 1, lesions 4-6).

These findings are in agreement with recent biochemical observations which show that the radioimmunoassayable enkephalin content of the basal ganglia does not change after cortical ablation¹⁴, but decreases by 50% following cell body destruction induced by intrastriatal injection of kainic acid⁴. They are also in agreement with the reported appearance of enkephalin immunoreactive cell bodies in the caudoputamen following inhibition of axonal transport by colchicine¹⁵.

Our results suggest that the Leu-enkephalin-containing nerve fibres in the pallidum do not belong to an intrinsic neuronal circuit but instead to a striopallidal pathway. Such a connection between the two basal ganglia nuclei is consistent with classical

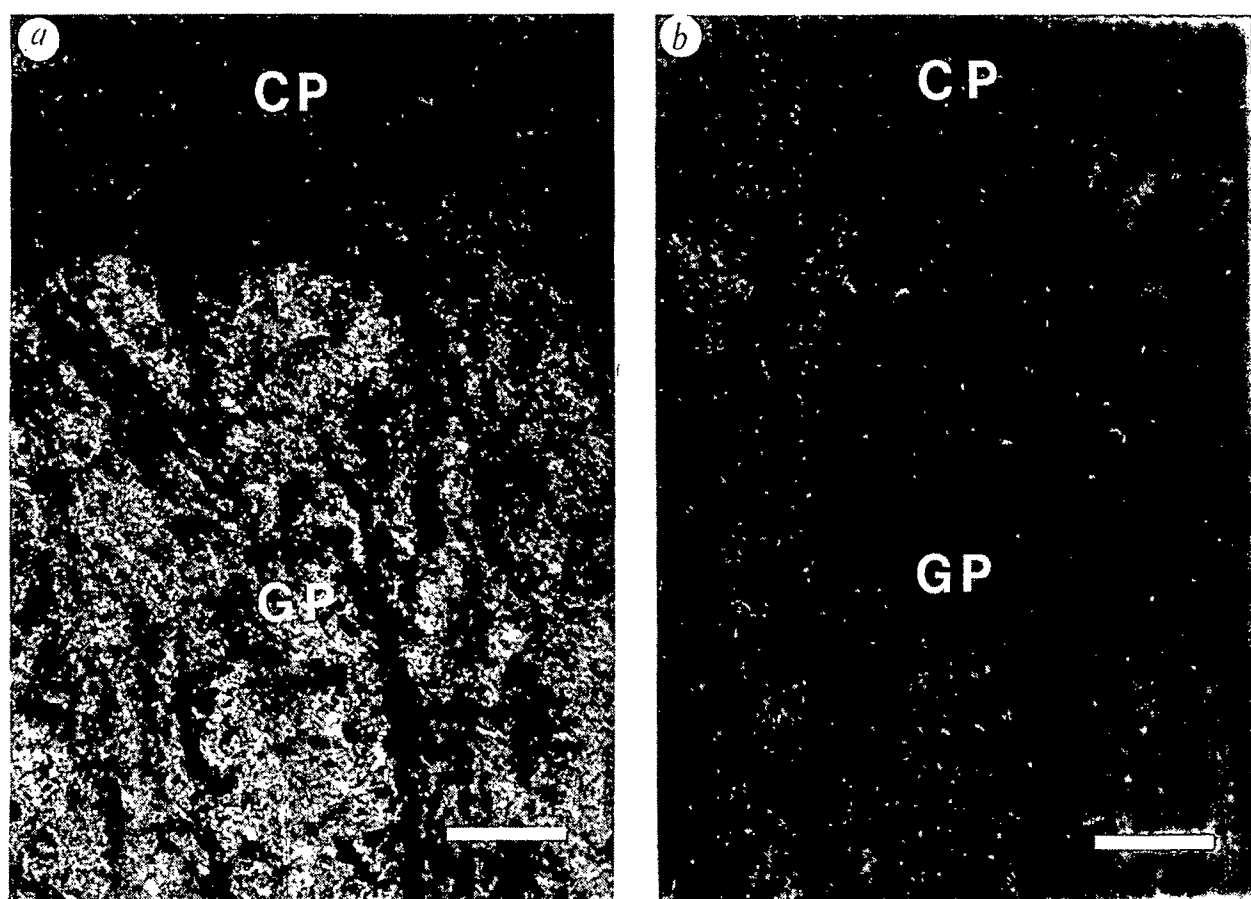


Fig. 2 *a*, Leu-enkephalin immunofluorescence in the globus pallidus (GP). Note the lack of immunoreactivity in caudoputamen. Horizontal section, untreated side. *b*, Ablation of Leu-enkephalin immunofluorescence in the globus pallidus following de-afferentation from the caudoputamen (contralateral to *a*). Scale bar, 225 μ m.

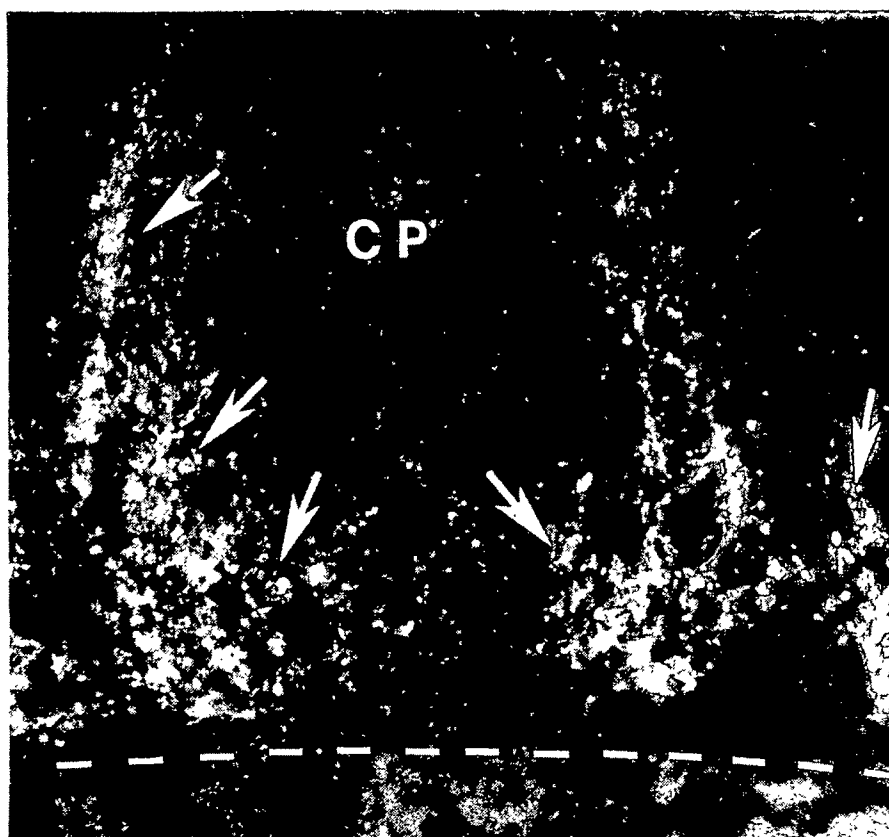


Fig. 3 Build-up (arrows) of Leu-enkephalin immunoreactivity in fibre bundles located in the caudoputamen (CP) at the level of the knife cut (dashed line). Scale bar, 40 μ m.

neuroanatomical studies which show neurones in the caudoputamen to branch extensively within the globus pallidus^{12,13}. The role of this 'long enkephalinergic' striopallidal pathway in extrapyramidal function and disease, remain to be established.

We thank Drs L. L. Iversen and J. S. Kelly for reading this manuscript, Mrs Angie Nelson for technical assistance, and Mr Chris Rayner and Mr George Marshall for their cooperation.

G.P. was supported by A.R.G.C. grant D 1-77/15750 (Australia).

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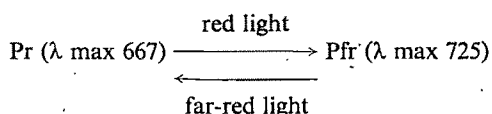
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Evidence for symmetry in the phytochrome subunit

PHYTOCHROME is a chromoprotein that has been shown to initiate and control major developmental responses of plants to light¹. It has two spectrally different forms that can be reversibly interconverted by light, as indicated:



The spectral properties of phytochrome are sensitive to changes in the microenvironment of the covalently bound chromophore(s). Relatively mild denaturing treatments, such as incubation at 25 °C for over an hour² and dehydration³, result in a significant loss of photoreversibility. In view of this susceptibility to spectral denaturation, it is remarkable that phytochrome can be proteolytically degraded to a molecular weight (MW) of 60,000, or half its native subunit size, without exhibiting any loss of photoreversibility or even any blue shift in its peak absorbance as Pr⁴. The additional fact that further proteolysis of phytochrome to 40,000 MW drastically reduces photoreversibility⁵ suggests that the 60,000 MW fragment has a sequence and configuration that are both sufficient and necessary to preserve photoreversibility. It is not clear whether this fragment is the product of one or more centrally located

cleavages of the native subunit to yield two pieces of MW near 60,000 per native subunit or whether it is the product of extensive proteolysis which yields a single 60,000 MW fragment per native subunit. We have compared the amino acid composition and the peptide map of the proteolytically-derived 60,000 MW fragment with those of the native 120,000 MW subunit. Our results strongly support the conclusion that the native 120,000 MW subunit is composed of two halves with nearly identical sequences.

To assure maximum purity, phytochrome was isolated from single bands on sodium dodecyl sulphate (SDS) gels after the preparative electrophoresis method of Stephens⁶. The samples used for the preparative electrophoresis were 70-80% pure and were isolated from etiolated oats by the method of Roux *et al.*⁷. By varying the extraction conditions this method can be used to produce both native phytochrome with intact 120,000 MW subunits and also the 60,000 MW fragment of phytochrome⁷. The MW of the smaller fragment was confirmed to be 60,000 by chromatography on a calibrated molecular sieve gel before electrophoresis on SDS gels. After the preparative electrophoresis, the purity and molecular weight of the samples were confirmed by re-electrophoresing aliquots of them on SDS gels. In all cases, 10 µg samples gave single bands at either 60,000 or 120,000 MW, indicating over 95% purity.

Table 1 compares the amino acid composition of the pure 60,000 and 120,000 MW phytochrome. This is the first report of the amino acid composition of the native phytochrome subunit. One analysis of each molecular weight species is shown. A second set of analyses on different phytochrome preparations that were also purified by the electrophoresis method gave identical results. To show that the preparative electrophoresis method of isolation does not produce composition artefacts and to further indicate the reproducibility of the results, the composition of 60,000 MW phytochrome isolated by standard chromatographic methods⁸ is given alongside of the electrophoretically isolated phytochrome.

The data in Table 1 reflect the composition values in mol % after subtracting the background values of a blank. This background contamination level was determined by amino acid analysis of material extracted from blank areas of the preparative gels. The reproducible contamination level was low but measurable and typical of that reported by others using the same technique⁹. The standard error limits for the final extrapolated values given are ± 0.2 mol % for all residues except Gly and Asp, where the error limits are about ± 1.0 and ± 0.5 mol % respectively. Thus the 120,000 and 60,000 MW peptides have statistically the same compositions at six residues (Lys, His, Arg, Ser, Met, and Ile). The differences at the other residues range from barely significant (Cys, Phe, Thr: 0.3 mol %) to 1.6 mol % for Gly.

The composition data are consistent with the hypothesis that the isolated 60,000 MW fragments were a heterogeneous mixture of two dissimilar halves of the native subunit. But they also suggest the possibility that the subunit may be composed of two nearly identical 60,000 MW regions. To distinguish which of these conclusions was more likely, tryptic peptide maps of the two phytochrome species were prepared. If the native subunit had little or no internal symmetry, then, ideally, one would expect to identify a number of tryptic peptides equal to the number of Lys and Arg residues in the native subunit plus one. On the other hand, the greater the symmetry, the fewer the number of different peptides that would separate on the map, with the lower limit being the number of peptides that were separable on the map of the 60,000 MW fragment. From the composition data, there were 59 lysines and 49 arginines per 120,000 MW subunit and 30 Lys and 24 Arg in the 60,000 MW fragment.

Complete tryptic digests of electrophoretically pure 120,000 and 60,000 MW phytochrome were prepared and chromatographed as described in the legend to Fig. 1. Two maps of two separately purified samples were made for both the intact

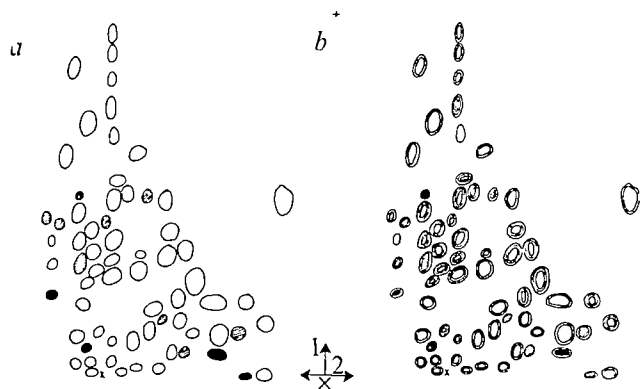


Fig. 1 *a*, Peptide mapping on thin-layer Silica G plates was patterned after a procedure by Stephens¹⁰. The isolated protein was subjected to complete tryptic digestion using a protein to DCC-trypsin ratio of 100:1 (w/w) for 24 h at 25 °C in 1% (NH₄)₂CO₃. The reaction was terminated by addition of several drops of glacial acetic acid followed by lyophilisation with three washes of distilled water. The digests were chromatographed on 20 × 20 cm Silica G plates in butanol-acetic acid-water (80:20:20) and air-dried overnight. High voltage electrophoresis in a perpendicular direction was performed on a Savant flat bed high voltage electrophoresis unit at 1,000 V for 105 min using pyridine-acetic acid-water (100:8:397), pH 6.5, solvent. The plates were oven-dried for 1 h at 110 °C. The final map was visualised with ultraviolet light after spraying with fluorescamine (0.025% in acetone). The fluorescing map was photographically documented using Kodak contrast process pan film. This figure represents a composite of six different peptide maps; three of the 120,000 MW subunit and three of the 60,000 MW fragment. The open circles denote those peptides common to both phytochrome species (55). Shaded circles denote peptides seen only on maps of the 120,000 MW subunit. Cross-hatched circles denote peptides only on maps of the 60,000 MW fragment. *b*, This is the same composite as (*a*) but the intensity of fluorescence is recorded. Three concentric circles denotes bright, two concentric circles denotes moderate, and a single circle denotes dim intensity.

subunit and its fragment. The peptide 'fingerprints' were visualised after fluorescamine treatment by their fluorescence under ultraviolet light. From photographic prints of the maps, *x-y* coordinates and relative intensity values were assigned to each peptide spot, and from these assignments the composite map shown in Fig. 1 was drawn.

The composite map illustrates that we were able to identify only 60 different peptides on the map of the 120,000 MW subunit, and of these 55 were in the same relative position and had

the same relative intensity as distinct peptide spots on the map of 60,000 MW phytochrome. As shown in Fig. 1, we identified 63 peptides on the map of the 60,000 dalton fragment, eight more than the ideal maximum predicted from the Lys and Arg content of the fragment. This anomaly can probably be ascribed to the likely size microheterogeneity of the 60,000 MW preparation. Such heterogeneity would be expected as this fragment is produced by the action of a variety of endogenous oat proteases¹⁰, none of which, presumably, are trypsin. Direct evidence of the heterogeneity of 60,000 MW phytochrome was provided by Rice and Briggs¹¹, who identified four different N-terminal amino acids in a preparation which was homogeneous in SDS gel analysis. The fact that two of the peptides unique to the 60,000 MW fragment only dimly fluoresce suggests that they are minor components of the population and further supports the hypothesis of microheterogeneity within the population of these fragments.

To rule out the possibility that some of the brighter spots represented multiple peptides whose fluorescent intensity masked their resolution as separate spots, we ran additional maps on reduced quantities of tryptic digests. Though these maps gave greater resolution of the brighter spots, they did not reveal any new spots. To demonstrate that our chromatography conditions would allow the separation of more spots, if they were present, we ran maps of phytochrome samples which were only 80% pure. We were able to identify up to 12 new spots on these maps, in addition to those depicted in Fig. 1.

The data presented above strongly support the hypothesis that the native subunit of phytochrome has extensive regions of internal symmetry in its primary structure. Because the compared amino acid compositions and peptide maps are not identical, it is unlikely that the symmetry is complete. These data do not show whether the symmetry is tandem or bilateral, nor do they reveal whether the symmetry extends to the number and position of the chromophore(s) of phytochrome. Our results contradict the conclusion inferred from immunochemical evidence that the 60,000 MW fragment of phytochrome bears little resemblance to the other half of the native subunit¹². But these data do not argue against the conclusion that the native subunit is, in fact, of MW 120,000. The evidence for the latter¹¹ seems incontrovertible.

It is not known whether the extensive sequence duplication in phytochrome has any functional significance. Information on this point may come from experiments in progress to compare the competence of 60,000 and 120,000 MW phytochrome to affect the properties of native¹³ and model¹⁴ membranes.

Table 1 Amino acid composition of 120,000 and 60,000 MW phytochrome

	Residues in mole %		Comparative data based on available 24-h hydrolysates†		
	Complete composition* 120,000	Complete composition* 60,000	60,000	60,000‡	60,000§
Cysteic acid	1.5	1.8	—	ND	ND
Aspartic acid	10.4	9.6	9.7	10.0	10.0
Threonine	4.2	4.5	4.3	4.3	4.4
Serine	7.3	7.5	6.8	6.8	6.5
Glutamic acid	11.4	10.2	10.9	10.4	10.0
Proline	4.6	5.6	6.0	5.6	6.1
Glycine	5.9	7.5	7.8	6.9	6.6
Alanine	8.8	9.3	9.4	9.6	9.7
Valine	7.4	6.7	6.6	7.1	6.7
Methionine	2.8	2.8	2.7	2.7	2.7
Isoleucine	4.9	4.7	4.8	5.1	4.8
Leucine	11.2	10.1	10.2	10.4	11.1
Tyrosine	2.2	2.6	2.5	2.6	2.8
Phenylalanine	4.1	4.4	4.5	4.5	4.6
Lysine	5.6	5.5	5.9	6.1	6.3
Histidine	3.0	2.8	3.1	3.0	3.1
Arginine	4.7	4.5	4.8	4.9	4.6

ND, not determined.

* Combined data from 24, 48, 72 h hydrolysates. Thr and Ser determined by linear extrapolation to zero time. Cys determined as cysteic acid after hydrolysis in the presence of dimethylsulphoxide according to Spencer and Wold.¹⁵

† Data compared only for phytochrome from *Avena sativa*, cv. Garry.

‡ Data from ref. 8.

§ Data from work of Fry and Mumford (see ref. 8).

We thank T. Cédel, G. Georgevich and K. McEntire for assistance in preparation of the protein. S.J.R. and W.E.B. acknowledge the support of the US NSF.

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Neutron diffraction studies on selectively deuterated phospholipid bilayers

NEUTRON diffraction combined with the use of selectively deuterated lipids can provide detailed information on the molecular structure of membranes. Because of the large difference between the coherent scattering length of hydrogen (-3.74 fermis) and deuterium (6.67 fermis) the deuterated membrane segments show up as intense peaks in the neutron density profile and can thus easily be located in the membrane¹⁻³. We have applied this method to bilayer membranes of 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine (DPPC), selectively deuterated at 12 different positions in the polar head group and the hydrocarbon chains. We report here that the mean position of the deuterated segments within the membrane can be determined in most cases to a precision of better than ± 1 Å. The average orientation of the phosphocholine group in the gel state as well as in the liquid crystalline state is almost parallel to the membrane surface. In the gel state the two hydrocarbon chains are out of step by about 1.8 Å, and water penetrates up to the glycerol backbone of the lipid molecules.

The selectively deuterated DPPC molecules were synthesised as described elsewhere^{4,5}. The measurements were carried out at low-water content (5-6 weight % water; 20°C) and at high-water content (25 wt % water; 28°C , 38°C , and 50°C). At low-water content the multilayered system was orientated on quartz slides and the Bragg reflections were measured in so-called $\theta-2\theta$ scans¹ on the D16 diffractometer at Institut Laue-Langevin (ILL). The mosaic spread measured for these samples had a half width at half height of about 12° . Figure 1a, b shows the first 10 lamellar orders for DPPC deuterated in both chains at the fifth carbon atom for the first sample and at the 15th carbon atom for the second. Only the first orders are very strong whereas the higher orders are so weak that most of them can hardly be seen in this plot. The quite dramatic differences in the ratios of the peaks on changing the label position indicate the sensitivity of the method. For the high water content samples, the lipid mixed with 25% water was sealed into cells with quartz windows. The resulting diffraction pattern consists of Debye-Scherrer rings which were observed with a two-dimensional position sensitive detector⁶ (the D11 camera at ILL).

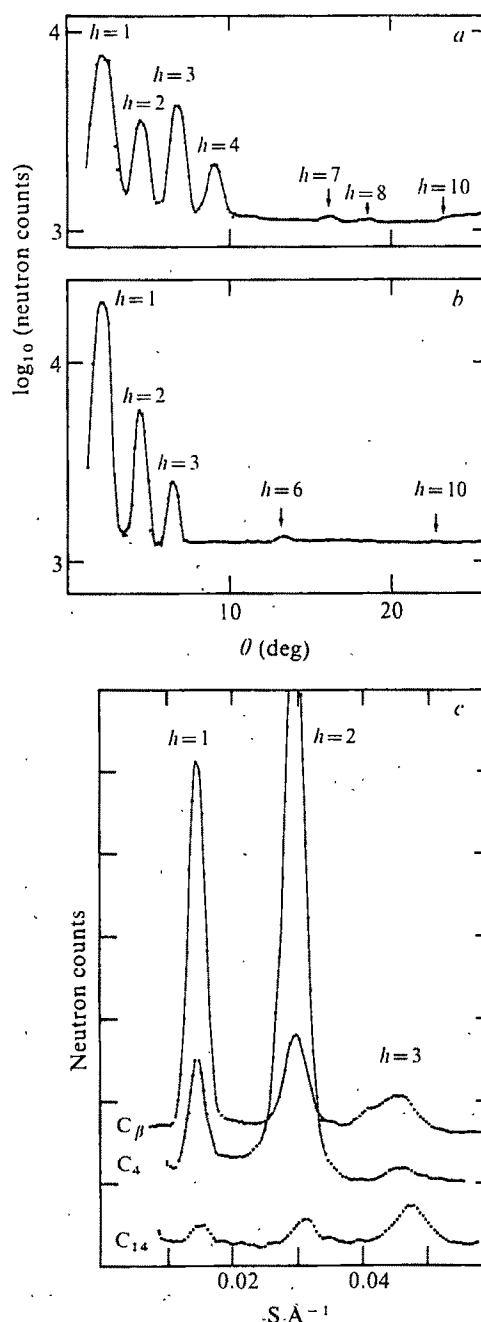


Fig. 1 Diffraction pattern of the lamellar reflections of DPPC multilayers. *a, b*, 10 orders from a $\theta-2\theta$ scan of two DPPC samples deuterated at the C15 (*a*) and C5 (*b*) position respectively. Each sample was orientated on quartz slides (water content 5-6% wt H_2O , 20°C , $d = 57.4$ Å, $\lambda = 4.43$ Å, θ = Bragg angle). *c*, Profiles of three Debye-Scherrer rings of powder samples deuterated at C_{β} , C_4 and C_{14} position respectively, as measured on D11 at ILL (water content 25% wt H_2O , 28°C , $d = 62.5$ Å, $\lambda = 6.26$ Å, $s = 2 \sin\theta/\lambda$). In order not to complicate the picture the three curves were shifted with respect to each other.

Because of inherent disorder in these samples the intensities of the diffraction orders become quite rapidly smaller with increasing scattering angle and it was difficult to observe more than four reflections. The first three orders, shown in Fig. 1c, illustrate the large changes in the ratios of the reflections when different segments of the lipid are deuterated. From these intensities, after appropriate corrections, the structure factors have been derived. Since the structure is centrosymmetric the phase problem is reduced to the choice between 0 and π . Compared to X-ray analysis, neutron diffraction offers several advantages for phasing and also for scaling the individual samples. Thus all the strong

orders are phased by using the isomorphous replacement technique where H_2O in the bilayer is successively exchanged against D_2O (refs 1–3).

Figure 2a and b contains the density profiles of DPPC/ H_2O mixtures labelled at the C15 and C5 positions and correspond to the diffraction patterns of Fig. 1a, b. Figure 2c is the difference profile of the C5-labelled lipid in D_2O and in H_2O showing the water distribution.

In principle, it is possible to obtain the position of the deuterium label directly from the density profiles; however, this procedure introduces some uncertainties because of Fourier series truncation errors. In addition, it would have been very time consuming to determine higher orders precisely for all the samples in different conditions (H_2O – D_2O –water content, temperature) as in most cases these orders are weak. Therefore we restrict ourselves to measuring only the strong reflections and to calculating the label positions in reciprocal space by the following procedure. After scaling and phasing each set of structure factors, the appropriate differences between pairs of different sets were derived. These difference sets were then used in a model fitting calculation in which the label distribution was assumed to be gaussian. Mean positions for the labels were therefore obtained with all 12 data sets contributing in each case.

Table 1 summarises the mean positions for the various deuterated segments of DPPC bilayers; the DPPC structure is given in Fig. 3. The distances are measured from the centre of the bilayer. The thermodynamic state of the bilayer is designated according to Luzzati and Tardieu^{7,8}. At low-water content (5–6 wt %) and 20 °C the bilayer is in the gel state and from the separation of the C2 segments of the fatty acyl chains the thickness of the hydrocarbon region is found to be 37.6 ± 2 Å. This result represents an average value, however, since it has been obtained from an experiment in which both fatty acyl chains were deuterated. Labelling the two chains individually reveals different distances for the two C2 segments. The C2(2) segment of fatty acyl chain 2 (attached at the C2 atom of the glycerol backbone) is about 1.9 Å further away from the centre of the bilayer than the corresponding C2(1) segment in the adjacent fatty acyl chain (attached at the C1 atom of the glycerol backbone). Furthermore, model calculations on the density profile of C15-labelled DPPC (Fig. 2a) also show that the two deuterated C15 segments of the same lipid molecule are separated in the bilayer by about 1.8 Å. These results then prove that the two hydrocarbon chains of DPPC in the membrane are out of step by about 1.5 carbon–carbon bonds with fatty acyl chain 2 shifted closer to the membrane surface. A similar finding has been reported for single crystals of dilauroyl-phosphatidylethanolamine⁹. On the other hand, the three segments of the choline moiety (C_α , C_β , C_γ) are positioned at almost

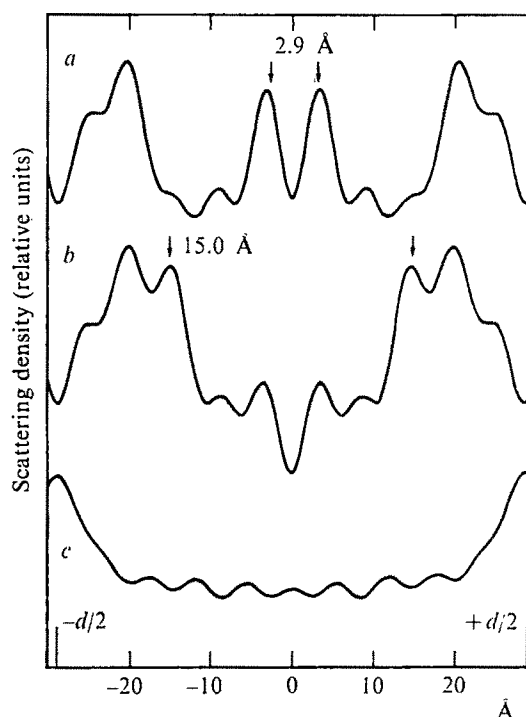


Fig. 2 6-Å resolution scattering length density profiles of DPPC at low-water content (5–6%) and 20 °C. a, deuterated at the C15 position; b, at the C5 position; c, water distribution determined from a difference profile of C5 in D_2O and H_2O . The lamellar spacing was 57.4 Å.

the same distance from the centre of the bilayer, which proves unambiguously that the phosphocholine dipole is orientated parallel to the membrane surface. This is further substantiated by comparing the experimental results with corresponding distances measured from a space-filling molecular model of DPPC (column 3). In this model the two fatty acyl chains were assumed to be out of step by 1.8 Å. Furthermore, in order to comply with the X-ray measurements⁸ both chains were tilted by 16° with respect to the bilayer normal. The choline head groups were either aligned parallel (column 3a) or extended perpendicular (column 3b) to the membrane surface. Table 1 shows that only the parallel head group orientation is in agreement with the experimental findings.

The last three columns represent the results at high-water content. In the L_β phase an increase in the water content from 5 wt % to 25 wt % changes the tilt angle of the hydrocarbon chains

Table 1 Summary of the mean label positions of DPPC in the membrane

	5–6 wt % water 20 °C L_β phase $d = 57.4$ Å	Distances in a space-filling model		28 °C L_β phase $d = 62.5$ Å	25 wt % water* 38 °C P_β phase $d = 62.2$ Å	50 °C L_α phase $d = 54.1$ Å
		head surface a	head ⊥ surface b			
Labels in the head group						
C_γ	25.1 ± 0.6	24.4	29.6	24.4 ± 0.6	24.5 ± 0.6	21.8 ± 0.6
C_β	24.8 ± 0.7	25.3	28.0	24.1 ± 1	23.9 ± 1	21.2 ± 1
C_α	24.5 ± 0.7	24.0	27.2	23.6 ± 1	23.0 ± 1	21.0 ± 1
GC3	23.1 ± 1.0		23.5	21.6 ± 1.5	21.4 ± 1.5	17.4 ± 1.5
Labels in the chains						
C2(2)	20.0 ± 1.0		19.4			
C2	18.8 ± 1.0		18.5			
C2(1)	18.1 ± 1.0		17.6			
C4	16.2 ± 0.6		16.1	15.4 ± 1.5	15.3 ± 1.5	12.2 ± 1.5
C5	15.0 ± 0.6		14.9	13.7 ± 1.5	12.2 ± 1.5	10.5 ± 1.5
C9	10.1 ± 1.0		10.1	9.8 ± 1	9.4 ± 1	8.1 ± 1
C14	4.1 ± 0.6		4.1	4.1 ± 1	3.6 ± 1	3.6 ± 1
C15	2.9 ± 0.6		2.9	2.5 ± 1	2.0 ± 1	1.9 ± 1

See Fig. 3 for structure of DPPC showing label positions.

*The three bilayer phases were also investigated by means of X-ray diffraction. The various patterns agree with those published by Janiak *et al.*¹⁰.

from 16° to 30° (ref. 10), leading to a concomitant reduction of the projected length of the hydrocarbon chains by 1.75 Å. This is also reflected in the position of the deuterium labels which in the high-water content L_{β} phase are shifted closer to the bilayer interior (column 4). At the pre-transition temperature of 35 °C the bilayer structure changes from L_{β} to P_{β} . According to Janiak *et al.*¹⁰ the P_{β} phase is characterised by a two-dimensional lattice with a rippled bilayer. The label positions as seen by neutron diffraction are almost the same in the L_{β} and P_{β} phase (column 5), however, and we therefore conclude that this effect produces only minor structural changes in the projection normal to the bilayer plane. It could be argued that the observed intensities of the I (h, 0) rings also contain contributions from the weak I (h, k) reflections which

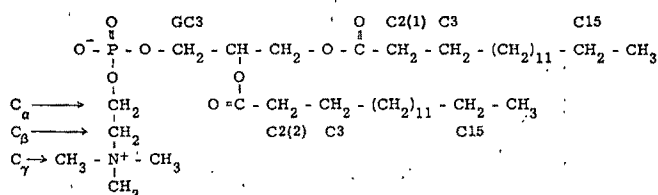


Fig. 3 Structure of DPPC molecule showing labelling positions (see Table 1).

would reduce the accuracy of our structure factors. Analysing the densitometer tracing of the P_{β} phase X-ray picture we can estimate a maximum error of 6% in the structure factors if three orders of I (h, k) are superimposed on one I (h, 0) reflection. This error does not seriously affect the determination of the mean label positions.

At 50 °C the DPPC bilayers are in the liquid crystalline state with disordered hydrocarbon chains. This is evidenced by a remarkable contraction of the DPPC bilayer by about 6–8 Å, but again the three segments of the choline moiety exhibit almost the same distance from the centre of the bilayer. Thus regardless of whether the membrane is in the gel or in the liquid crystalline state and independent of the water content the phosphocholine dipole is always parallel to the plane of the membrane. We are at present investigating the effects of ions on the head group structure.

We thank Dr H. Stuhmann for helping us to start this work and Dr V. Luzzati for his support. We thank Dr D. Schneider for a program for handling the D11 data. This work was supported by an EMBO travel grant (G.B.) and by grant 3.008.76 from the Swiss NSF (J.S.).

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Proximity of two tryptophan residues in dihydrofolate reductase determined by ^{19}F NMR

NUCLEAR magnetic resonance methods have provided a wealth of information on the conformational and ligand-binding properties of proteins^{1–3}. In such studies, it is necessary to resolve resonances from the ligand or from individual residues of the protein. To achieve this, it is often necessary to resort to isotopic substitution methods such as selective deuteration^{4,5} or ^{13}C enrichment^{6–8}. An alternative approach is to introduce a fluorine atom (^{19}F) into the system as an NMR probe, either by using fluorine-containing ligands^{9–11} or by labelling the protein itself. This can be done either by chemical modification^{12–15} or by the biosynthetic incorporation of selected fluorinated amino acids^{16–19}. We have used this latter approach to prepare *Lactobacillus casei* dihydrofolate reductase containing either 3-fluorotyrosine or 6-fluorotryptophan residues; by measuring the ^{19}F spectra at 94.1 MHz we have characterised the effects of ligand binding on individual tyrosine and tryptophan residues in the protein¹⁹. We now report through-space ^{19}F – ^{19}F spin–spin coupling in 6-fluorotryptophan containing dihydrofolate reductase which demonstrates that two of the tryptophan residues are in close proximity in the folded structure of the protein.

Figure 1a shows the ^1H noise-decoupled ^{19}F NMR spectrum at 94.1 MHz of 6-fluorotryptophan-containing dihydrofolate reductase in its ternary complex with methotrexate and NADPH. Each of the five fluorotryptophan

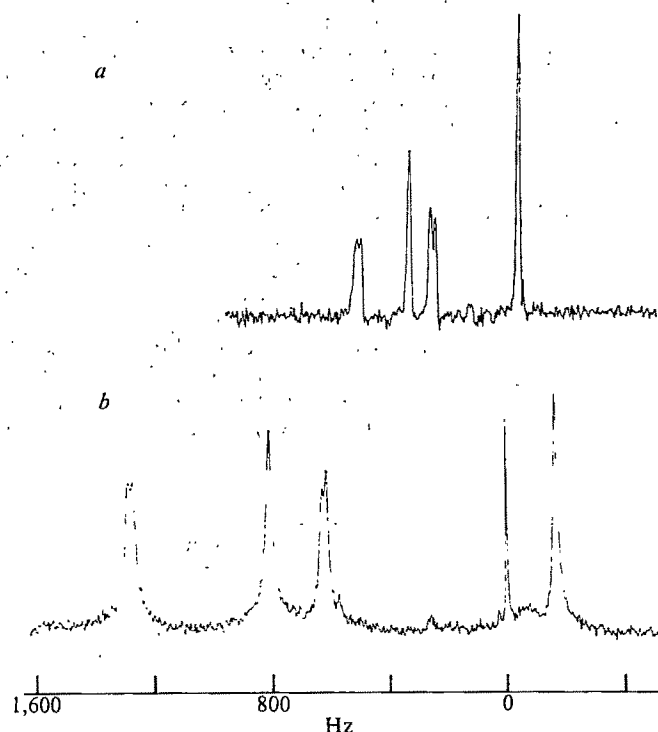


Fig. 1 a, The proton noise-decoupled ^{19}F NMR spectrum at 94.1 MHz of a 1 mM solution of 6-fluorotryptophan-labelled dihydrofolate reductase in the presence of 1 equivalent of methotrexate and 1 equivalent of NADPH. The sample was examined as a D_2O solution containing 50 mM potassium phosphate, 500 mM potassium chloride, pH 6.5 (meter reading); sample temperature was 11 °C. Details of the purification of the enzyme and experimental conditions used for recording the spectrum are given in ref. 19. The high field signal has been assigned to two overlapped ^{19}F signals; however, more recent sequence studies (K. Batley and H. R. Morris, personal communication) suggest that the enzyme contains only four Trp residues. b, The proton noise-decoupled ^{19}F NMR spectrum of the same sample at 254 MHz (sample temperature 28 °C). The additional sharp signal at zero frequency arises from a small amount of denatured enzyme which appears on standing.

residues gives rise to a ^{19}F NMR signal. It can be seen that two of the signals appear as doublets, with components of approximately equal intensity and with splittings of equal magnitude (17 ± 2 Hz). Clearly, since all the ^1H nuclei are being irradiated, these splittings cannot arise from ^1H - ^{19}F spin-spin interactions. The two remaining possibilities are, first, that the splitting is a spin-spin coupling, which must be a ^{19}F - ^{19}F coupling since there are no other spin- $\frac{1}{2}$ nuclei present a 100% abundance in the system or, second, that the splitting is a chemical shift difference, reflecting the presence of two equally populated conformational states of the protein. These two possibilities can be distinguished either by spin-decoupling ($\{^{19}\text{F}\}-^{19}\text{F}$) or by studying the field-dependence of the splitting, since chemical shift differences are directly proportional to the strength of the applied magnetic field, while spin-spin couplings are field independent. We have now examined the ^{19}F spectra at 2.7 times higher field strength (254 MHz for ^{19}F) and find that the splittings are again 17 ± 2 Hz, that is they are independent of the operating frequency and thus correspond to spin-spin interactions and not to chemical shift differences. The constancy of the splittings can be clearly seen in Fig 1a and 1b where the spectra at 94.1 and 254 MHz are presented on the same frequency scale. Instrumental limitations have prevented us from carrying out the triple resonance experiment (^1H and ^{19}F irradiation) required to demonstrate ^{19}F - ^{19}F homodecoupling. However, we have been able to confirm that the doublets arise from homonuclear ^{19}F - ^{19}F spin-coupling by studying the ^{19}F spectra obtained using Carr-Purcell pulse sequences ($\pi/2-\tau-\pi-\tau$)²⁰. In these experiments the singlet echo resonances decayed as a function of $\exp(-2\tau/T_2)$ whereas the doublet echoes decayed as $\exp(-2\tau/T_2) \cos(2\pi J\tau)$ as would be expected for doublets arising from homonuclear spin coupling. When $2\tau=1/J$, inverted signals are observed for such a doublet while the singlets are not inverted. An analysis of the amplitude of the high field doublet signal (see Fig. 1) as a function of τ gives $T_2 = 38 \pm 8$ ms and $J_{\text{FF}} = 21 \pm 2$ Hz.

We therefore conclude that we are observing, for the first time, ^{19}F - ^{19}F spin-spin coupling between the nuclei on different tryptophan residues in the protein.

Such coupling is termed 'through-space' coupling, since there is no inductive mechanism available. The concept of through-space coupling is well-established in small molecules²¹⁻²⁶ where the interacting nuclei are close together in space though separated by four or five σ bonds. For such through-space coupling to occur the fluorine atoms must be sufficiently close together ($<4\text{\AA}$) for there to be significant orbital overlap²²⁻²⁶. As the internuclear separation decreases to 3Å coupling constants of approximately 20 Hz have been observed and further decrease in the internuclear distance leads to a sharp increase in the magnitude of the coupling constant. We conclude that two fluorotryptophan residues in *L. casei* dihydrofolate reductase must be so disposed that fluorine substituents are approximately 3Å apart (approaching van der Waals contact). Since no two tryptophan residues are adjacent in the primary structure of the protein (K. Batley and H. R. Morris, personal communication), this proximity must be a consequence of the folded structure of the protein. (It is apparent in Fig. 1b that the resonance of the denatured enzyme shows no splitting.) The measurement of through-space ^{19}F - ^{19}F spin coupling constants clearly provides us with a sensitive method of detecting conformational changes in this part of the protein. In a series of complexes with substrate, coenzyme or inhibitors (alone or in combination) the magnitude of the splitting was completely unchanged, even though the chemical shifts of these two ^{19}F signals changed by up to 1 p.p.m.¹⁹. Thus, although the environment of the two tryptophan residues changes, their relative position does not.

The observation of through-space ^{19}F - ^{19}F coupling in proteins opens up an entirely new source of information on protein structure and protein-ligand interactions in solution. A systematic study of a series of selectively fluorinated derivatives of a protein could provide much valuable information on the proximity of individual residues to one another in the protein. The principle of the method could readily be extended to studies of interactions between fluorine-containing ligands and fluorine-containing proteins. The appearance of through-space coupling between a nucleus on the ligand and one on the protein is entirely feasible, and would provide unique information on the structure of the binding site in solution. Through-space ^1H - ^{19}F coupling also occurs²⁶, and would open up further possibilities for the study of protein-ligand interactions using a fluorine-containing ligand and a normal protein. It should be noted that spin-coupling constants can be detected only in the spectra of low-molecular weight proteins: for higher molecular weight proteins ($>30,000$) the short relaxation times preclude such measurements.

The information obtained from observations of through-space coupling is similar to that which can sometimes be obtained from studies of the nuclear Overhauser effect^{27,28}, and a combination of the two approaches should provide structural information on proteins in solution. We plan to explore the potential of these methods fully in our studies of ^2H - and ^{19}F -labelled dihydrofolate reductase.

B.D.S. acknowledges the financial support of the MRC of Canada Group on Protein Structure and Function. We thank Dr L. H. Sutcliffe for helpful discussions, and the Wellcome Foundation for financial support to B.B.

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matters arising

Molecular chirality of life and intrinsic chirality of matter

BONNER *et al.*¹ stated that if racemic DL-leucine was bombarded with left-handed electrons (-1 helicity) plus their bremsstrahlung, the D enantiomer decomposed more quickly, whereas with right-handed electrons ($+1$ helicity) plus their bremsstrahlung the L enantiomer decomposed faster. In an aqueous system of D- and L-tyrosine and using only natural, left-handed β^- particles and their bremsstrahlung radiation I found² similar results in 1968. Bonner *et al.*¹ referring to my experiment, wrote: "One of us has been unable to repeat Garay's original type of experiment". They used the word 'repeat', erroneously. Bonner² certainly repeated parts of my experiment for his paper but he used only bremsstrahlung radiation and left out β^- particles from the reaction mixture. This altered the situation fundamentally and therefore it cannot be considered repetition. Bonner *et al.* discussing their results, state: "We do not know whether the asymmetric degradations are caused by the longitudinally polarised electrons or by their bremsstrahlung"¹. Thus the presence of longitudinally polarised electrons are clearly important. According to our hypothesis^{8,9} and to a similar one put forward by Noyes³, longitudinally polarised electrons are responsible for stereoselective degradation. Additionally, Keszthelyi^{4,5} and Walker⁶ pointed out that selective decomposition of enantiomers cannot be caused by bremsstrahlung. Finally, in recent experiments, Darge *et al.*⁷ studied the stereoselective decompositions in a similar system. They changed the radiation source and target molecule, but did not omit β^- particles from the reaction mixture. Referring to my work they wrote that their result "is in qualitative agreement with previously reported experiments on the interaction of D- and L-tyrosine with β^- particles".

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BONNER, VAN DORT AND YEARIAN
REPLY—Garay quotes incompletely from our paper¹ on the asymmetric degradation of DL-leucine, then claims that we use the word "repeat" erroneously. We said¹, "In addition, one of us has been unable either to repeat Garay's original type of experiment with alkaline DL-tyrosine or to observe the induction of optical activity in a number of other racemic amino acid substrates as well". Neither in this quote nor in the paper to which it refers² do we claim an attempt to repeat Garay's exact experiment. In fact, we elaborate in detail on the reasons for which we modified Garay's experimental protocol, because² "we have felt some slight misgivings about Garay's experiment. In the first place the β -source was relatively weak, only about 0.36 mCi of $^{90}\text{SrCl}_2$. Second, the intended reaction was not primarily induced by the β -rays or their bremsstrahlung, but only presumed to be in some way enhanced by them. Thus the effect was noted only in alkaline, but not acidic solution. Third, the reaction was conducted in aqueous-ethanol solvent, where solvent photochemistry or radiochemistry (which would involve symmetrical radical intermediates) would be superimposed upon and might obscure any actual asymmetric β -ray or bremsstrahlung effects. Lastly, we felt that mere differences in the shapes of two ultraviolet absorption spectra were hardly the best or most convincing criteria for the generation of optical activity. For these reasons we decided to repeat Garay's type of experiments, with considerable modifications which would hopefully circumvent some of the above drawbacks." Since the Vester–Ulbricht β -decay mechanism³ which we were investigating involves β -ray bremsstrahlung, we used² the strongest bremsstrahlung source we could locate, 61.7 kCi of ^{90}Sr – ^{90}Y oxide in storage at Oak Ridge National Laboratory. To minimise other sources of ambiguity inherent in Garay's original experimental design we irradiated both racemic as well as optically-active amino acids, both crystalline and in solution, then analysed the percentage degradation and enantiomeric composition of the radiolysed samples by gas chromatography. Clearly, these experiments represented extensions of and not a repetition of Garay's original experiment. In none of these experiments was asymmetric degradation observed. Our subsequent suc-

cessful asymmetric degradations of DL-leucine with longitudinally-polarised linear accelerator electrons of both handedness¹ involved crystalline amino acid targets in a collodion matrix under high vacuum, and certainly also do not remotely approximate Garay's original experimental conditions. Nor do the recent experiments of Darge⁴ involving the radiolysis of DL-tryptophan by ^{32}P in a frozen ice matrix constitute a duplication of Garay's experiment. The simple fact is that no one, including Garay, has made an attempt to repeat his original experiment exactly, and no one has claimed to.

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Do viruses use calcium ions to shut off host cell functions?

DURHAM¹ suggests that Kamine and I² have been "misled into putting false emphasis" on the role of Mg^{2+} relative to Ca^{2+} in controlling cellular metabolism because we have been unduly influenced by the fact "that cells respond more to changes in external Mg^{2+} than Ca^{2+} ". This represents a ordinate control of metabolism and misreading of my reasons for proposing a central role for Mg^{2+} in the co-growth of animal cells. These reasons were set out at greater length previously^{3,4} and have been reiterated and expanded more recently (see ref. 5 for example). My emphasis on a regulatory role of Mg^{2+} stemmed from the observations that any of a variety of unrelated substances which stimulate the multiplication of chicken embryo fibroblasts in culture, also stimulate a singular array of reactions associated with transport⁶, energy metabolism⁷, differentiated function and macromolecular synthesis^{8,9}. This singular array was termed the coordinate

response^{3,5} and includes reactions which are independent of one another and of macromolecular synthesis. Therefore, it was necessary to invoke an intermediary agent between external signal and intracellular response with the capacity to modulate many different metabolic pathways at the same time. The only specific enzymatic steps then known to be accelerated by the stimulatory treatments were the transphosphorylation reactions of glycolysis^{7,10}, all recognised as control points in this pathway and all requiring Mg^{2+} (refs 11–13). It has also become evident that some key regulatory enzymes vary in activity with their degree of phosphorylation^{14,15} and that the activities of the protein kinases or phosphatases which determine this degree are sharply dependent on $[Mg^{2+}]$ in the physiological range. In a slightly different cellular domain, we have shown that the rates of uptake of hexoses and uridine into chicken embryo cells, which are governed coordinately by external effectors, can be controlled by Mg^{2+} , but not by Ca^{2+} , in a manner that simulates in detail the kinetics of the physiological response¹².

It is generally agreed that over 90% of the Mg^{2+} in cells is bound, largely to membranes and macromolecules^{17–20}. Changes in configuration of the binding structures or in their microenvironment would alter the availability of Mg^{2+} for its metabolic tasks. Increasing the permeability of the cell membrane to Mg^{2+} would also tend to drive up the intracellular concentration of Mg^{2+} (ref. 21). Because the estimated concentration of free intracellular Mg^{2+} is less than that required for maximal activity of many key regulatory enzymes^{11–14} any small change would have far reaching effects²².

By contrast, the concentration of free Ca^{2+} in cells is far too low²³ to affect the regulatory enzymes of the pathways involved in the coordinate response^{3,5}. Perhaps some mechanical responses which involve proteins with a very high affinity for Ca^{2+} are under its control. But, the remarkable sequestering power of the cell for Ca^{2+} (ref. 24) would ensure that such a response would be short-lived, unlike the coordinate response which is maintained for many hours, requires continuing stimulation, and culminates in accelerated cell division.

There would seem to be some merit in Durham's suggestion that the inhibition of cell metabolism which accompanies infection by cytotoxic viruses is caused by a gross increase in intracellular Ca^{2+} following damage to the cell membrane. This follows from the observation that an internal Ca^{2+} level, $[Ca^{2+}] \geq 10^{-4} M$ interferes with key Mg^{2+} -dependent reactions in the cell^{25,26}. Beyond such pathological

effects, however, the role of Ca^{2+} seems likely to be restricted to short-term structural and mechanical responses in keeping with the pulse-like, localised nature of its fluctuations, and to the limited number of proteins which can respond to $[Ca^{2+}]$ within the physiological range of $<10^{-7}$ – $10^{-3} M$. Long-term inhibition of metabolism by Ca^{2+} deprivation may be the indirect result of lowering free Mg^{2+} within the cell⁴.

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DURHAM REPLIES—The best current estimates of ion concentrations in resting eukaryotic cytoplasm are around $10^{-3} M$ for Mg^{2+} and $10^{-7} M$ for Ca^{2+} . This 10^4 -fold difference explains why Ca^{2+} and not Mg^{2+} is used as a short-term intracellular signal in movement, hormone action, synaptic transmission, protozoan chemotaxis, vision and bioluminescence. Powerful homeostatic mechanisms tend always to maintain very low cytoplasmic Ca^{2+} concentrations. Prolonged overwhelming of these mechanisms would produce a spectrum of changes strikingly like those produced by many lytic or transforming viruses.

Rubin and his colleagues have shown clearly that changes in extracellular Mg^{2+} concentrations can greatly affect cells. Others have shown analogous responses to extracellular K^+ or H^+ levels, and to agents that affect polyamine metabolism. Responses to extra-

cellular Ca^{2+} are notoriously variable, however, even for processes that undoubtedly involve intracellular Ca^{2+} . One reason is that eukaryotic cells conduct most Ca^{2+} fluxes across internal membranes, to and from substantial calcium reservoirs for which there are probably no magnesium equivalents. In the long term, feedback relationships between different ions tend to obscure the primary ion fluxes, so that one is probably wise not to make categorical statements about any one ion.

Rubin's statement that the cytoplasmic Ca^{2+} concentration is too low to affect the "coordinate response" is wrong. Micromolar Ca acts on adenyl and guanyl cyclases and phosphodiesterases, with consequent effects on cyclic nucleotide levels and kinase activities. It also acts on K^+ and other ion fluxes, and on DNA precursor synthesis. Rubin's implication that intracellular Ca^{2+} ions act via Mg^{2+} -dependent reactions can be only partly true.

Rubin and I agree that cell biologists frequently postulate, and then expensively seek, macromolecules to fill roles that ions can fill much more simply.

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Cholinergic link in yawning

HOLMGREN *et al.*¹ recently focused attention on the basic mechanism behind the act of yawning. They reported that physostigmine and pilocarpine induce yawning in young male rats and hypothesised that a central cholinergic link may be involved in the reflex. Our results support such a proposal.

Yawning is a characteristic sign of withdrawal from morphine in man² and monkeys³. When naloxone (0.5 mg per kg body weight), but not physiological saline, was injected subcutaneously (s.c.) into three 'ex-addict' baboons (two male and one female, 4.4–5.2 kg), 98 d after abrupt withdrawal of morphine, a low incidence of yawning (2–4 episodes) occurred within 15 min; on this occasion, other signs of long-term withdrawal were absent. Seven days later, the same baboons were again challenged with naloxone. 20 min after physostigmine (0.05 mg per kg s.c.). Although this dose of physostigmine *per se* did not elicit yawning, with each animal there was a threefold increase in the incidence of yawning.

Although a cholinergic link may indeed be involved in yawning, it should be recognised that other factors are also important. Thus, dimethyltryptamine causes

yawning after intramuscular administration to rhesus monkeys⁴ yet there is no evidence that this hallucinogen has marked effects on the cholinergic system in this species.

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URBÁ-HOLMGREN AND HOLMGREN
REPLY—In Cowan's experiments with 'ex-morphine addict' baboons, although physostigmine 0.05 mg per kg s.c. *per se* did not evoke yawning, this dose strongly potentiated naloxone-induced yawning. Perhaps a higher dose of physostigmine (0.10 mg per kg) would have elicited yawning directly even in monkeys, as it does in rats¹ and in infant guinea pigs, kittens and dog pups (unpublished observations). In infant rabbits we have had to use a still higher dose (0.15–0.20 mg per kg).

We certainly agree with Cowan that other factors are also important in yawning. The stretching and yawning syndrome induced by ACTH and MSH (quoted in ref. 1) is a well-known example. In recent experiments with (3,4 dihydroxyphenylamino)-2-imidazoline (DPI, Böhringer) we have observed that this drug, which according to Cools *et al.*² has a specific and potent agonistic activity at dopamine inhibitory receptors, in doses of 5 mg per kg intraperitoneally, elicits moderate, but statistically significant yawning in infant rats (from 9 to 15 d in age). It would be rash at this stage, to hypothesise that drugs shown to elicit the yawning act necessarily through a final common path including a cholinergic link.

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Lysogeny by f2 phage?

ZGAGA¹ claims to have produced "the first demonstration that bacteria can also contain the genetic information for the production of an RNA phage", and raises the question whether RNA phages can lysogenise their host in certain conditions. Her criteria are the "spontaneous release of

phage particles" and the "immunity" of the host to superinfection.

Davern², and Hoffmann-Berling and Mazé³ reported that *Escherichia coli* infected with RNA phage (can₁ or fr, respectively) when incubated at 32°C rather than 37°C seemed to grow normally while producing phage at a low level. The suggestion that single infected cells excreted phage without lysing³ has been challenged⁴. Davern further noted that even at 37°C phage-infected cultures showed variable degrees of lysis and the surviving cells seemed to multiply normally while producing phage indefinitely in successive cycles of subculture. Such cells, when used as host bacteria in a plating assay, did not permit formation of plaques. The latter observations have been repeated several times in our laboratory when indicator bacteria have been accidentally contaminated with phage.

The state in which bacterial cultures produce phage without lysing and are resistant to superinfection has been called 'carrier state'² or 'viral persistence'⁵ without implying any mechanism. Resistance to lysis occurs in certain physiological states of *E. coli*⁶ and resistance to superinfection (interference) has been described as a normal consequence of RNA phage infection⁷. The phenomena described by Zgaga are reminiscent of the earlier observations and raise the question whether guanidine is required to establish cultures of bacteria "containing genetic information for f2 biosynthesis".

The term 'lysogeny' implies that the viral genome is present in the host in a non-infectious form, in particular that it is integrated into the host genome⁸; until evidence for such criteria is adduced in the case of RNA phages it would be preferable to retain the original designation of 'carrier state'.

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ZGAGA REPLIES—Weissmann's objection is based on the observations of Davern¹ and of Hoffmann-Berling and Mazé², that *E. coli* infected with some RNA phages apparently grow normally at lower temperatures while producing phage at a low level. Loeb and Zinder³ showed that at 37°C f2 phage lyse the bacteria they infect. Lerner and Zinder⁴ showed, by the study of release of bacteriophage f2 from single infected cells, that at 30°C as well as at 37°C phage are released by cell lysis,

and not by excretion from intact cells. Therefore, the definition of 'carrier state' mentioned by Weissmann ("the state in which bacterial cultures produce phage without lysing and are resistant to superinfection") obviously cannot be attributed to phage f2.

Originally, 'carrier state'⁵ denotes "bacterial cultures that are persistently contaminated with phage, but from which uninfected cells could be recovered readily". Thus, the maintenance of this state depends on the presence of free phage and sensitive bacteria. To eliminate any possibility of reinfection during bacterial growth, female (*con. f2*) cells, which are genotypically resistant to f2 phage, were constructed. It turned out that F[–] (*con. f2*) bacteria produce phage spontaneously. In addition, 'carrier state' is not stable. Therefore phage-free, sensitive clones can usually be isolated by growth in the presence of phage antiserum, or by serial subcultures of single colonies⁶. On the contrary, the principal feature of 'lysogeny' is its stability. Hayes said⁷: "In practice, the existence of lysogeny should be judged by rather rigorous criteria of its stability, since interaction of some virulent phage with their hosts may superficially simulate the condition" ('carrier state'). (*Con. f2*) state, once established, has been stable for 2 yr. It is stable in male as well as in female cells and quite unaffected by serial subculture of single colonies or by antiserum treatment.

The functional state and the location of genetic information for phage production in (*con. f2*) bacteria are still under investigation. But, I do not agree with Weissmann's notion that the term 'lysogeny' in particular implies, that the viral genome is integrated into the host genome. For example, in lysogens carrying phage P1, the prophage does not occupy a characteristic site, if any, in the bacterial chromosome⁷, but there is strong evidence to suggest that it is a nonchromosomal element⁸, and a part of the bacterial membrane replicatory system^{9–11}.

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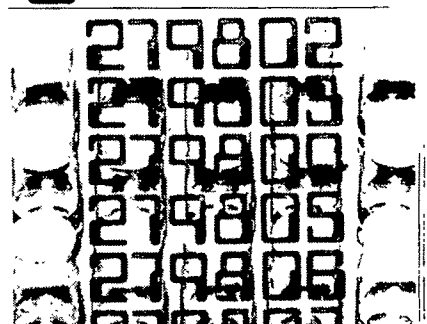
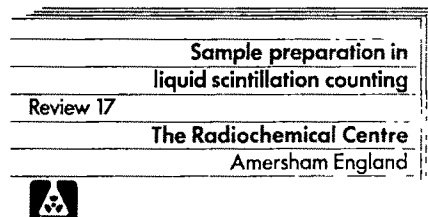
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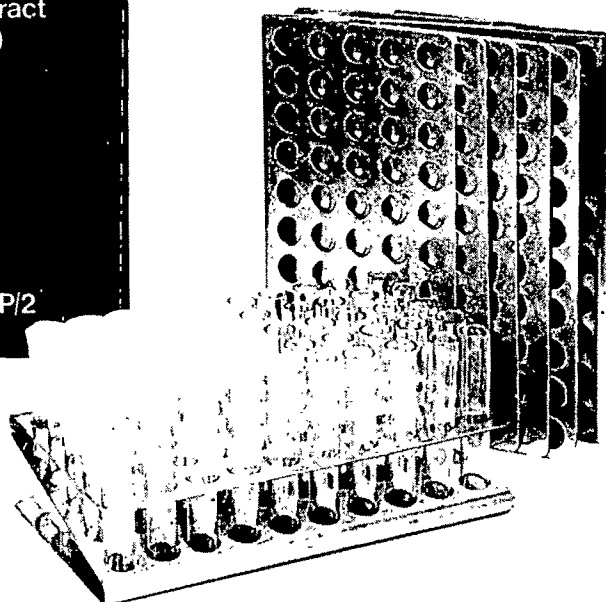
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reviews

Astronomy of Stonehenge

Jack Meadows

On Stonehenge. By Fred Hoyle. Pp. v+128. (Heinemann Educational: London, 1977.) £4.50.

SOME time ago, I acted as External Examiner at another university, and wanted to interview one of the candidates. She was nowhere to be found. Further enquiries elicited that she had recently become a devotee of Stonehenge; and I had been foolish enough to select the summer solstice for my oral examinations. She had chosen to attend the more important event on Salisbury Plain. I wondered then, not for the first time, what it is about Stonehenge that sets it apart in people's minds from other megalithic monuments. This even extends to discussions of its possible astronomical significance. After all, Thom has suggested that a number of the standing stones up and down the country indicate an astronomical orientation, so why do at least half of all relevant writings concentrate on Stonehenge?

Respectable reasons can certainly be advanced—the impressive size of the monument, the constructional capabilities that must have gone into its creation, the choice of site, the span of time over which it was used and modified. I suspect, however, that part of the cause of Stonehenge's attraction, even for reputable investigators, remains its extra-scientific glamour. Typically, Sir Fred Hoyle has his own different reason for studying Stonehenge:

"Because of my interest in Stonehenge, I am often asked to comment on other ancient monuments—Avebury, the Egyptian pyramids, Mayan temples, and so forth. It usually causes surprise when I express no interest. My indifference comes from a lack of definition of the problem. Given the plan of Avebury, it would be possible to invent many hypotheses about the purpose of this monument, so many indeed that soon or later, just at random, the investigator would come on something which seemed to have a tolerable correspondence with the facts. For me, the case of Stonehenge was quite different. Thanks to the work of Mr Newham and Professor Hawkins, the problem here is defined, particularly if we go on to add, as Professor Hawkins also did, the further assertion that the purpose of Stonehenge was to predict eclipses of the Sun and Moon. The problem is then defined with severe constraints. Without modifying the fixed structure in any

way, can Stonehenge be used in a literal, practical way to predict eclipses successfully? Since the successful prediction of eclipses is a complex problem, an affirmative answer to this question would certainly not be expected unless eclipse prediction was indeed the purpose of Stonehenge. The question is so definitive, permitting no room for manoeuvre, that its investigation is quite explicit."

There speaks the Cambridge-trained theoretician: we know precisely where we are (even if it is a little harsh to imply that no other site has yet had its problems properly defined).

Hoyle's book therefore has a single, limited purpose—to examine the possibility that Stonehenge was used as an eclipse predictor—and the author deploys his considerable skill as a writer in presenting his arguments and conclusions clearly and concisely. The main body of the text, though closely argued, uses no mathematics, and should be comprehensible to a wide range of readers. (The diagrams, incidentally, are genuinely helpful.) An appendix re-defines the problem in terms of spherical trigonometry; and also brings us face to face with one of the main queries concerning astronomical orientations—namely, their statistical significance. Two types of question come under this heading. The first is this: since alignments are never perfect—due, for example, to subsequent movement of the stones and to their finite breadth—what latitude of error can be permitted when deciding whether two objects are aligned towards a particular point on the horizon? Again, if a large number of possible alignments can be found (as they can at Stonehenge), what is the likelihood that astronomically significant ones will occur by chance? Hoyle examines both these points with care. He concludes with regard to the second, that the probability of chance alignments is higher than Hawkins supposed in *Stonehenge Decoded*, but is still too small to explain all the observed alignments at Stonehenge.

The discussion of Stonehenge's use as a predictor of solar and lunar eclipses essentially follows lines that Hoyle has put forward previously, though laid out here in greater detail. In particular, he spends some time considering the vexed question of how exactly observations of objects on the

horizon might have been made. This is linked to the discussion of alignment errors; since both the Sun and the Moon show a finite disc. His argument here is ingenious. He suggests that some of the apparent spread in alignments at Stonehenge, as compared with the expected directions, is due to the method of observation that was used. As a result, evidence previously used to cast doubt on an astronomical interpretation of Stonehenge, may actually support it. It is only fair to add that some of the details of the observational procedure he outlines may be open to question. The general picture, however, hangs together very well, and leads on to an interesting speculation regarding the successive stages of construction at Stonehenge.

One of the problems in interpreting Stonehenge as an astronomical observatory is that the first structure built (Stonehenge I) seems to indicate a more sophisticated approach than the final stage (Stonehenge III). This obviously runs counter to common-sense expectations. Hoyle suggests that, in the period between the two phases of building, the method of eclipse prediction changed. Stonehenge III has an inner horseshoe of 19 bluestones, and he relates this to the Saros—the 19-yr cycle that was used in antiquity to predict eclipses. Recognition of the Saros, however, almost certainly required access to written records; whereas the analysis behind Stonehenge I could have been carried out on the basis of a simple counting system. On this interpretation, Stonehenge III does not reflect a simpler understanding of eclipses than Stonehenge I: rather, the complexity has been removed from the structure and stored in another form. A numerate culture became a literate culture.

The way we interpret Stonehenge necessarily affects what we think of the people who built it. Archaeologists currently seem somewhat less concerned about the level of abstract thought implied by a Stonehenge astronomical observatory than they were a decade ago. Nevertheless, the cultural implications continue to be controversial. In the final chapter of the book, Hoyle adds his own mite to this debate. He suggests that the (invisible) nodes of the lunar orbit, which he believes

were tracked by way of the Aubrey holes at Stonehenge, might have come to be worshipped as a powerful, though unseen, god. He therefore concludes the book by querying: "Could a distant memory of [the Sun, Moon and lunar nodes] be the origin of the doctrine of the Trinity, the 'three in one, the one in three'? I suspect so. In Stonehenge I may well be many roots of our present-day culture."

No doubt the student who stood me up for Stonehenge will feel confirmed in her choice by these words. Those who would not wish to venture so far should, nevertheless, be grateful to Sir Fred Hoyle for setting out the case for an astronomical interpretation of Stonehenge in such a readable manner.

Jack Meadows is Professor of Astronomy and History of Science at the University of Leicester, UK.

Microscopic techniques

Analytical and Quantitative Methods in Microscopy. Edited by G. A. Meek and H. Y. Elder. Pp. 276. (Cambridge University: Cambridge, London and New York, 1977.) Hardback £12; paperback £4.75.

THIS volume originates in a symposium on principles, applications and shortcomings of some techniques in modern microscopy, organised under the auspices of the Society for Experimental Biology in 1975. It consists of an introduction and twelve chapters. The topics considered are stereology, optical diffraction analysis, quantitative fluorescence microscopy, image analysis, integrating microdensitometry, scanning microinterferometry, STEM, microanalysis of various sorts, and cryoultramicrotomy.

Most of the chapters are written in a clear instructive style which is fairly easy to follow, although some of the authors have lapsed on occasion into the more easily produced review type of text. The editors have done well to cajole a panel of authors of this size into producing a reasonably uniform style and standard of presentation. The chapters seem to have very few statements to which one could take specific objection, although it is difficult for one person to critically encompass accounts of all of these topics. Each of the chapters is at such a level and of

a style as to make it very suitable first reading for a research student or a more senior researcher contemplating working in one of these areas of technique. Such readers will get a helpful mechanistic accounts of the methods concerned. The chapters by R. W. Horne (optical diffraction analysis), D. J. Goldstein (microinterferometry and microdensitometry) and S. Bradbury (quantitative image analysis) are particularly helpful and timely accounts.

Perhaps it should be asked how fair a picture this book gives of microscopy today. Taken together, they describe methodology that finds use at present in a small minority of research projects. The overall picture of microscopy presented in this volume is one that emphasises certain (sometimes quite isolated) specialities. It is for the most part about the iced cakes of microscopy rather than the bread and butter.

Perhaps the ultimate test of a book that is designed for the teaching of a technique is to see if it can tell the researcher whether a particular approach is appropriate for answering the questions he wishes to ask. I am not certain how many of the chapters pass this test, but there are some really excellent helpful accounts of certain microscope technologies in this book. The paperback version, in particular, is fine value.

M. A. Williams

M. A. Williams is Reader in Human Biology and Anatomy at the University of Sheffield, UK.

Insect behaviour-modifying chemicals

Chemical Control of Insect Behaviour. Edited by H. H. Shorey and J. J. McKelvey, Jr. Pp. 414. (Wiley-Interscience: New York and London, 1977.) \$24.50; £14.65.

THIS book is the eighteenth publication in the series *Environmental Science and Technology*, and deals with a subject that, because of its likely contribution to a more rational use of insecticides, is of particular

relevance to the study of the environment and the technology of its conservation. All types of behaviour-modifying chemical known to influence insect behaviour by external reception, including pheromones and plant-derived compounds, are covered and their prospects in pest management are discussed.

Publication of the book has arisen from a conference, involving many leading workers in the field, held in May 1975 at Bellagio, Italy, with participants contributing authoritative chapters on the various aspects of the subject. After a short introduction, subsequent chapters are arranged into five sections. The first deals with the nature of sensory responses to

behaviour-modifying chemicals with many examples from electrophysiological studies. The next two sections are devoted to behavioural aspects and begin with a detailed account of the various mechanisms by which insects respond to distant odour sources. The fourth section considers the role of diversity in behaviour-modifying chemicals, with the approaches to their use in insect pest management reviewed at length in the final section. Each chapter has an extensive list of references including many publications from 1975. The book has a comprehensive subject index.

The present high level of research activity in chemical control of insect behaviour may mean that this book, dealing with the subject in such depth, will soon become out of date. Already, for example, the treatment of chemokinesis in chapter 5 has been revised by the author, and in chapter 9 a point is made based on a recently questioned report that the sex pheromone of *Musca domestica* consists of one compound. Nevertheless, since the authors have, with only a few notable exceptions, been cautious in drawing conclusions from the data presented and are mostly in a position to judge this data critically, the likelihood of serious reappraisal of much of the content is greatly reduced.

As the book is compiled from many individual contributions, apparently without too rigorous editing, there is inevitably some duplication of information, and in some cases the treatment of such data seems to be inconsistent. This often presents the reader, however, with the opportunity to compare the various current approaches to controversial issues. For example, the subject of kairomones is treated in several different ways, and a number of authors make different suggestions for improving definitions of the terms that have been devised to fulfil the requirements of the subject.

The prospects for insect behaviour-modifying chemicals in pest management are considered critically and without the rash claims typical of many earlier writings. In addition, a number of realistic suggestions for new approaches are made although it is asserted that more rapid progress would be made in pest management if priority could be given to fundamental research in certain listed areas.

There is no doubt that this book is to be recommended. It will be of most value to those already having some knowledge of the subject and will provide a stimulating background on which to base new research. In addition, those engaged in deciding priorities for future crop protection research and development will find this book a source of useful information and ideas.

J. A. Pickett

J. A. Pickett is Principal Scientific Officer co-ordinating chemical aspects of work on behaviour-controlling chemicals at Rothamsted Experimental Station, Harpenden, UK.

applied excellence

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	[G-³H]VINBLASTINE Code TRK.507 17 44% 11' 8% 12, 13 46% 14' 2%
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Steroids vol. 28 (3), p.p. 359-375, 1976.

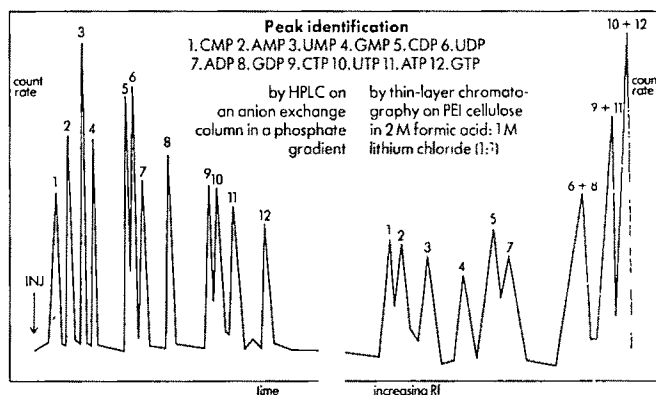
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Chemically transmitting synapses

Synapses. Edited by Glen A. Cottrell and Peter N. R. Usherwood. Pp. xviii+385. (Blackie: Glasgow, 1977.) £13.95.

THERE is a neatness and economy about the structure and function of chemically transmitting synapses that has given them an unfailing appeal for experimenters. The action potential, a brief all-or-nothing event, invades the presynaptic nerve terminal; the transmitter, synthesised and packaged on the spot, is released in quantal, countable packets; it combines with specific post-synaptic receptor sites which (in some situations at least) act to open or close discrete ionic channels in the membrane; the transmitter is then either metabolised on the spot by an enzyme appropriately lodged in the synaptic cleft, or it is taken back up by the presynaptic nerve for re-use. The whole thing seems to have an almost Toy-town quality.

To the extent that good science consists in reducing the confusion of a

half-understood mechanism to a series of discrete steps, this quality of our present understanding of synaptic function is a testimony to the excellence of past work in this field, and the casual observer might think that there was little left to be discovered. He would be wrong, of course, and this book, which is made up of a series of papers presented at a symposium in March 1976, highlights very usefully just those areas where mysteries still abound; in doing so, it obviously has to take for granted a great deal, and should be read as an introduction to the growing points and vague edges of the subject rather than as a coherent résumé of the present state of knowledge.

Among many interesting papers is a series of contributions about the vesicle hypothesis, written mainly by sceptics, and these constitute a very valuable source of information for students of this tangled controversy. It is a pity, perhaps, that papers by some of the ardent defenders of the vesicle hypothesis, such as Whittaker or Heuser, were not included.

Another fascinating theme that runs through several excellent papers in this volume is that of the formation and stabilisation of synapses, which is approached through studies on the synthesis and degradation of receptors (an

elegant contribution by Fambrough and his colleagues), the regeneration of synaptic connections after sectioning nerve bundles in the leech nervous system (Nicholls *et al.*), and studies on muscle denervation (Guttmann). This is surely one of the most challenging problems in neurobiology, and when we consider how badly understood is the mechanism even of the much-studied effect of denervation on the distribution of receptors in skeletal muscle fibres, it is obvious that the exploration has barely begun. The Toy-town image of the finished article seems a long way away when one is faced with the problem of how the synapse develops and regenerates.

Inevitably, in a published symposium, there are inconsistencies of style and quality. Overall, though, a very high standard is achieved, and the editors are to be commended for their choice of authors. Whether much purpose is served by including 40 pages of brief abstracts as an appendix to the main part of the book is doubtful, but this is a small criticism of an otherwise excellent book.

H. P. Rang

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Laboratory handbook on lysosomes

Lysosomes: A Laboratory Handbook. Second edition. Edited by J. T. Dingle. Pp. xiv+323 (North-Holland: Amsterdam, New York, and Oxford, 1977.) Dfl.136.00; \$55.50.

OF all the articles in the multi-volume *Lysosomes in Biology and Pathology*, edited by Dingle and his colleagues, those on techniques have been most frequently consulted. Dingle therefore decided to edit a laboratory handbook on lysosomes, which has proved sufficiently popular to justify a second edition.

The most useful chapter is a scholarly account of the properties of lysosomal enzymes by A. J. Barrett and M. F. Heath. This has been brought up to date with methods of assay and much background information. The chapter on isolation of lysosomes (R. T. Dean) follows for the most part traditional lines, using loading of rat liver with Triton, dextran, iron or gold. Methods for isolation of lysosomes from single cell types, such as leucocytes and tumour cells, are also given.

L. B. Bitensky and J. Chayen describe the histochemical identification of lysosomes at the light microscopical level, and rely on microdensitometry for quantitative results. The identification of lysosomes at the electron microscopical level is discussed by J. M. P. Schellens and his colleagues. Attractive results are obtained in various cell types by techniques for carbohydrates such as silver proteinate. Even reactions for enzymes such as acid phosphatase or β -glucuronidase can be misleading. Observations must therefore be approached with caution.

During the past five years, specific antibodies to individual lysosomal enzymes have been studied by A. R. Poole and others to monitor purity of isolated enzymes, inhibit their activity in cells and tissues and localise individual enzymes within cells.

In general, this is a fairly comprehensive account of recent techniques for lysosomes and their constituents, well illustrated and produced. It will be as welcome and widely used as the first edition.

A. C. Allison

A. C. Allison is Head of the Division of Cell Pathology at the MRC Clinical Research Centre, Harrow, UK.

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Applications of geomorphology

Applied Geomorphology: A Perspective of the Contribution of Geomorphology to Interdisciplinary Studies and Environmental Management. Edited by John R. Hails. Pp. xv+418. (North-Holland: New York, Amsterdam and Oxford, 1977.) \$39.95; Dfl.98.00.

THE descriptive models of landscape evolution written in the sonorous prose of the last century were debated but only a little refined over 50 years, with too little attention being paid (though there were exceptions) to the mechanics of the processes invoked in sculpting type forms of the land surface. In comparatively recent years, enormous strides forward have been made, largely by application of quantitative methods in the laboratory and in the field. The subject is becoming established as a science in its own right, and though still interdisciplinary between civil engineering, geology and geography, the applied aspects compel its inclusion in optimisation of land use, in building science, and in pedology.

The subtitle of the book truly indicates the contents, written by nine authors, each an acknowledged leader in his field and (as in the editor's introduction) able to "communicate ideas comprehensible to others who have no geomorphological knowledge".

Some authors discuss widely, as in the chapter on weathering, with applications ranging from ore deposits to geomedicine, whereas others present thorough yet succinct summaries of their discipline; a very creditable example of this is the chapter on "Periglacial Environments". The chapter on irrigation and ground-water studies demonstrates how the success of irrigation schemes would be enhanced if designed to fit the geomorphic units compiled from interpretation of the geomorphic history of the area. Processes controlled by climatic differences have less importance in Karst hydrology, as the author of that chapter makes quite clear; he also cites Sweeting's view that the study of the development of Karst landforms has probably been hindered less by the dominance of cyclic ideas than by the lack of contact of workers in the different Karst regions. From work initially conceived in a spirit of academic enquiry, there is now much of importance to the engineer involved in dam building or using limestone aquifers as reservoirs or quarries for waste disposal.

The chapter on fluvial hydrology explains the relation between some of the many variables to be measured in

comprehending the river as an integration of the catchment morphology. Man's use and modification of rivers has often proved needlessly expensive in corrective measures applied where these measures opposed the natural forces.

The same can be said of the problem of routing motorways, with examples in the chapter on soil mechanics of slopes. The degree of stability of previously stable slopes cannot yet be predicted.

It seems paradoxical in the chapter on deserts to see the stress on removal of sediments by water: though widely spaced in time, these processes are critical in terrain classification and environment.

For resource appraisal, trafficability and management problems, systems of terrain classification, with subgroups of land units, are explained using block diagrams, maps and tables to help comparison of different methods and applications.

A chapter on coastal zone planning and management includes sea defences,

off-shore banks and beach stability, and discusses how these can be affected by tourist use, by mining and by construction works.

Inevitably, the great range of style and subject matter compels dipping into the book for a synopsis of specific aspects; but the 20 pages of index are inadequate for the reader looking for specific topics across different chapters—for example, rates of rock wear or removal by different geomorphic processes. The 35 pages of references are very valuable and would have been doubly so if the page number could have been added to indicate where referred to in the text.

There are numerous maps, tables and graphs and nine half-tone plates, leavening a most valuable summary of some quite difficult branches of this global science.

H. Lister

H. Lister is Reader in Physical Geography at the University of Newcastle upon Tyne, UK.

Differentiation in cellular slime moulds

Development and Differentiation in the Cellular Slime Moulds. Edited by P. Cappuccinelli and J. M. Ashworth. Pp. xx+317. (Elsevier/North Holland: Oxford, New York and Holland, 1977.) Dfl. 79; \$39.75.

THIS volume is a collection of papers from an EMBO workshop held in April 1977. One is grateful that it has been published so quickly, but this advantage is somewhat diluted by the way in which the book is printed. The typewritten manuscripts have been directly photocopied and greatly reduced; as a result some of the papers are either so faint or the print so small that they require hard work to read. What with the high price of the volume, the least the publishers could have done is to issue a magnifying glass with each copy, like the compact edition of the Oxford English Dictionary.

Cellular slime moulds are having a burst of popularity, and this excellent collection of papers gives one a good view of current progress. Many of the papers are mixtures of reviews and new results, some of the latter being significant advances.

To show where the main interests lie at the moment, I have made a list of the areas covered in the symposium: six of the papers are on some aspect of differentiation; five on cyclic AMP pulsations and their various effects in-

cluding their role in chemotaxis; four studies which in some way relate to problems of transcriptional and translational control of protein synthesis; three on different aspects of genetics; two on the plasma membrane; and one each on cell contact, cell division, polyamines, and modelling biochemical pathways.

One theme that runs through a number of papers is the rôle of cell contact on different aspects of development. J. Gross *et al.* found that cell attachment during aggregation, the rate of cyclic AMP signalling and the appearance or disappearance of certain proteins, all change at the same time. H. V. Rickenberg *et al.* show that cell contact is not necessary for the synthesis of alkaline phosphatase in cells grown in liquid culture if the suspension of separate cells is pulsed with cyclic AMP. H. F. Lodish and T. H. Alton provide evidence suggesting that at early stages when cells are separate, protein synthesis is controlled at translation, since there are numerous mRNAs present which are not translated. At aggregation, however, transcriptional control takes over and all the mRNAs are directly used to make new protein.

It is fitting that this interesting collection of papers should be dedicated to Kenneth Raper who discovered *Dictyostelium discoideum* and led the way in showing the exceptional usefulness of cellular slime moulds as experimental organisms.

J. T. Bonner

J. T. Bonner is Professor of Biology at Princeton University, New Jersey.

obituary

J. E. Littlewood

PROFESSOR John Edensor Littlewood, FRS, Emeritus Rouse Ball Professor of Mathematics in the University of Cambridge, died on 6 September, 1977, at the age of 92.

He was the son of Edward Thornton Littlewood, a schoolmaster who had been 9th Wrangler in 1882. Littlewood was taught mathematics at St. Paul's School by F. S. Macaulay, later FRS, an outstandingly successful teacher. Littlewood has described his mathematical education, including his early experiences in research, in *A Mathematician's Miscellany*, and it seems to have been a far from ideal preparation for the kind of research which became his life. For Cambridge mathematicians had not yet fully appreciated the major advances on the Continent.

His director of studies, E. W. Barnes, suggested the problem of integral functions of zero order, in particular asymptotic formulae for functions with given zeros. Perhaps as a result of independent reading, Littlewood switched to 'elementary' methods for general functions and produced an important result in his first paper on the maximum and minimum moduli of any integral function of zero order, a type of result which Barnes had described in 1898 as 'a disguised truism'. However, Barnes now recognised the value of the work; one referee opposed publication violently, but G. H. Hardy reported favourably and the paper was published in 1907.

In 1911, Littlewood opened a new chapter in the theory of series with his Abel-Tauber theorem which led to his immensely important collaboration with Hardy lasting 35 years.

It covered, besides the theory of series, and in particular Fourier series, the Riemann zeta function, Diophantine approximation, the additive theory of numbers and the theory of functions.

After three years in Manchester, Littlewood returned in 1910 to a Fellowship and Lectureship at Trinity College; he became Rouse Ball Professor in 1928. During the First World War he worked as a Second Lieutenant on anti-aircraft ballistics. He retained his rooms in Trinity as Life Fellow until his death. He spent little of any vacation in Trinity, finding a different setting better for concentration on research. He believed that periods of complete relaxation were essential and often found them in music, and in rock-climbing and skiing.

In his younger days he served on the Councils of his College, the Royal Society and the London Mathematical Society, but from about 1935 the fits of depression which had plagued him for many years seem to have become worse. He took no part in meetings of committees or councils or in gatherings of mathematicians. However, his fits of depression were cured sometime in the 1950s, and he paid several highly successful visits to the U.S.A. after retiring.

A very high proportion of Littlewood's work after 1912 was done in collaboration with Hardy, some with Paley, Offord, myself and others. When Harald Bohr, brother of Niels Bohr, paid his first long visit to Cambridge, he and Littlewood wrote a monograph on the zeta function, but, when it was finished, they were so exhausted that they could not take it to the printers. The manuscript was used later by Titchmarsh and Ingham

in writing their Tracts on the subject. Of the papers appearing under Littlewood's name alone, the one in 1925 made a very important contribution to the problem of the coefficients of functions *schlicht* in the unit circle, a problem still not completely solved.

Although in the early days there must have been many discussions with Hardy, most of their joint work was done by correspondence. It seems that there was an unwritten agreement that Hardy could write up and publish anything based on joint work, but Littlewood only allowed me to write up parts of our joint work on condition that it appeared under my name alone as 'based on joint work.' My own collaboration with Littlewood arose out of a D.S.I.R. memorandum on the nonlinear differential equations arising in radio work, and the most important result arose out of a letter to *Nature* 120 (1927), 363, by van der Mark and van der Pol on frequency demultiplication, or rather van der Pol's interpretation of it in *Proc. Inst. Radio Engrs* 22 (1934), 1051-1086.

I used to go to Littlewood's lectures on the theory of functions; some of them are included in his book of that title, and at one time he used the galley proofs. Although he had given the same lecture two or three times before, he showed such a passionate interest, always seeing things from a slightly different angle, that his audience could not help sharing his enthusiasm.

Littlewood's display of unequalled insight, technique and power as an analyst, was recognised by honorary doctorates, medals and membership of leading foreign academies.

Mary L. Cartwright

W. H. Sheldon

DR WILLIAM H. SHELDON, Director of the Biological Humanities Center in Cambridge, Massachusetts, died there on 16 September 1977. Sheldon is the man who invented somatotyping—a classification of variations in human body structure, to which he related variations in temperament, in physical and mental illness, and in patterns of growth and aging.

Born in 1898 in Warwick, Rhode Island, Sheldon grew up amid woods and marshes. His work shows the imprint of his country background; of his mother who raised five children and

was midwife to a village; of his father, a naturalist, breeder and judge of animals; and of his godfather William James, the psychologist and philosopher. At age 10 he was working for the state ornithologist, reporting on animals of the woods and fields—observing, describing, classifying. At 15 he took pride in his ability to match judges' scorings of livestock on 100-point scales. As an adult he turned his naturalist's eye on human structure and behaviour.

After public school, Sheldon attended Brown University and the University of Colorado. From the University of

Chicago he received his Ph.D. in psychology in 1926, his M.D. in 1933. He taught at Northwestern University and the Universities of Chicago and Wisconsin. A two-year fellowship in Europe allowed him to study with Jung and to visit Freud and Kretschmer.

In 1938 he moved to Harvard, where he did much of his basic research, with such colleagues as S. S. Stevens, the experimental psychologist, and E. A. Hooton, the physical anthropologist. During World War II he served as lieutenant colonel in the Army Medical Corps. From 1947 until 1959 he was Director of the Constitution Labora-

tory at the College of Physicians and Surgeons, Columbia University, succeeding Dr G. Draper, a pioneer in constitutional medicine. He has also held research appointments at the University of California, Berkeley, and, since 1951, at the University of Oregon Medical School.

Sheldon's earliest book (*Psychology and the Promethean Will*, 1936) and his latest one (*Prometheus Revisited*, 1975) are broad, provocative and provoking schemes for merging religious humanism with biologically grounded social psychiatry. Between these publications, he worked singlemindedly to propose and refine methods for describing individual human physical structure: to develop a "biological identification tag". Best known are his primary components of endomorphy (roughly speaking, the softness and roundness of a physique), mesomorphy (heaviness of bone and muscle development), and ectomorphy (attenuation, "stretched-outness"). By measuring the strength of each component in each individual and assigning a three-part index, the somatotype, Sheldon produced a tool which comes much closer to describing and encoding the great range of varia-

tions on the basic human body plan than was possible with older, pigeon-holing typologies.

Over the years his methods moved through several revisions, toward objectivity and always seeking measures that maximise invariance, that evaluate physique not just as a current manifestation but as a lifelong trajectory. Along the way he has reported associations of somatotype with temperament, delinquency, and mental illness. His reports on physique, health, and longevity, followed in the surviving veterans of the Spanish-American war (1898), and on the later careers of 200 delinquent boys first studied 25 years ago, will be completed by his colleagues.

Sheldon's studies of some biological underpinnings of behaviour offered a much-needed counterbalance to a psychology one-sidedly emphasising learning and environment. Psychology regarded this offer doubtfully, though the term *somatotyping* found currency in labelling many sorts of physique appraisal methods, much as the term *psychoanalysis* was misapplied to all sorts of appraisal and treatment methods, and even "schools" of

somatotyping developed, like the schools of psychoanalysis (Sheldon found this comparison odious).

A keen observer, Sheldon was thoroughly convinced of the value and accuracy of his observations—an asset in a pioneer researcher, a problem to academic psychologists mistrustful of personal judgments. His irreverence for some of psychology's sacred cows, and his schoolboy's sense of mischief combined with a talent for finding just the right parallel in nature to point up a description of a human structure or action, have needed many a colleague. But Sheldon enjoyed his life. A seven-days-a-week worker, a diner at cafeterias because he could not stand the delays and pretentiousness of restaurants, an inveigher against the poisons in modern life, he was also wholehearted in his appreciation of an idea, a friend, a sparrow on his windowsill, an old coin. (Numismatists know him as the author of the standard work classifying early American cents.) His work cleared and broadened one roadway into the study of human biology and behaviour that had been an overgrown trail.

Richard N. Walker

Arthur C. Hardy

ARTHUR C. HARDY, Professor of Physics at the Massachusetts Institute of Technology and inventor of the first recording spectrophotometer, died on 31 October at the age of 81.

His specialist interests were in optics and photography, and after graduating at the University of California in 1917, he immediately became involved in photography in the first world war when he served in the American Expeditionary Force as a Commanding Officer in the Photographic Section of the U.S. Army. After the war he spent two years at the Kodak Research Laboratories before taking up an appointment in 1922 as Assistant Professor in Optics and Photography at MIT, where he spent the remainder of his professional life, becoming successively Associate Professor, full Professor and, in 1961, Emeritus Professor.

He played some part in the development of sound recording on film, while in 1930, in collaboration with Professor S. F. Brown, he developed an electric organ which was intended to reproduce the sound of any musical instrument. It is, though, with his famous recording spectrophotometer that his name will always be associated, since it is fair to say that this instrument revolutionised industrial colour measurement.

Until then, spectral transmission and reflection measurements were made with visual instruments and the observations were extremely laborious and were often impossible because of

shortage of light. The recording of a spectral reflection curve through the visible spectrum in the course of only a few minutes meant that it now became a commercial proposition for the colour industries to determine the key property of their products, namely the spectral absorption of a dye or pigment.

The instrument has really had a very remarkable history. It was developed in the 1920s at MIT and a fascinating account of how the design evolved was given by Professor Hardy in 1938. (*History of the Design of the Recording Spectrophotometer*, *J. opt. Soc. Am.* **28**, 360–364; 1938.)

A number of the MIT staff participated in the project and a major element in its success was the use of an optical attenuator, a train of polarising prisms, to balance the light reflected from the sample against the light reflected from a reference white. The photoelectric cell, which was in those days a rather uncertain device, could therefore be relegated to the role of a null detector. In more recent recording spectrophotometers, on the other hand, the measurement is usually obtained by comparing the photocurrents themselves using a ratio-recording potentiometer or similar device. Yet the Hardy instrument is still holding its own against all competitors and is still regarded by many users as the standard instrument for colour measurement.

It was produced commercially by the American General Electric Company

as the G.E. Spectrophotometer, but it might have had an early rival from Westinghouse, since in 1930 I was involved, as a research engineer with Westinghouse in Pittsburgh, in discussions about the production of a competitor. These were not, however, followed up. It is now manufactured by the Diano Corporation as the Diano-Hardy Spectrophotometer and, naturally enough, includes a number of modifications and accessories although the basic design is still the same after nearly 50 years.

A further contribution which Professor Hardy made to colour measurement was the publication in 1936 of the *Handbook of Colorimetry*, prepared under his direction by a team drawn from the staff of MIT. This was the first book on colorimetry to appear following the establishment, by the Commission Internationale de L'Eclairage, of the 1931 system of colour specification based on tables defining the colour-matching characteristics of a standard observer. The many charts and tables in the Handbook provided an excellent introduction to the CIE system and undoubtedly speeded up the use of the system for colour standardisation and as a tool in the colour industries.

Professor Hardy received a number of honours, including the Frederick Ives Medal of the Optical Society of America. He also served as President of the Society from 1935 to 1937 and as Secretary from 1940 to 1957.

W. D. Wright

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PLANT BIOLOGIST (26) Ph.D., with 1 year postdoctoral, tropical experience seeks U.K. based position. 970(B)

APPOINTMENTS VACANT

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swansea**

Chair of Geography

The Council of the College invites applications for the appointment of a Professor of Geography from August 1, 1978, or as soon as possible thereafter, consequent upon the retirement of Professor W. G. V. Balchin.

Further particulars may be obtained from the Registrar/Secretary, University College of Swansea, Singleton Park, Swansea SA2 8PP, to whom applications (10 copies) should be returned by February 28, 1978. 976(A)

UNIVERSITY OF SOUTHAMPTON FACULTY OF MEDICINE HUMAN REPRODUCTION AND OBSTETRICS

Applications are invited for the permanent post of Experimental Officer in Human Reproduction and Obstetrics. The person appointed will be expected to be in charge of the laboratories and to undertake a wide range of interesting experimental projects for the Unit. Candidates should have practical laboratory experience in Biochemistry/Endocrinology and research experience to Ph.D. level will normally be required. Salary scale: £2,904 to £4,811 (under review). The initial salary will depend on qualifications and experience.

Further particulars may be obtained from Mr. C. W. L. Swann, The University, Southampton SO9 5NH (to whom applications (4 copies from United Kingdom applicants) should be sent not later than January 27, 1978. Please quote ref: 85/A/N. 965(A)

TECHNICIAN

required in the Department of Oral Immunology and Microbiology for one year in the first instance with some experience in immunology and/or microbiology for research into the immune responses to microbial agents in immunodeficient children. Salary for qualified Technician not less than £2,982 plus £354 London Weighting.

Apply in writing, stating age and giving details of qualifications and experience, to the Secretary, Guy's Hospital Medical School, London Bridge, SE1 9RT, quoting Ref. I.M.5. 973(A)

UNIVERSITY OF HULL SCIENTIFIC ASSISTANT

Applications are invited for the position of RESEARCH ASSISTANT to participate in research on the role of the thymus in embryonically induced transplantation tolerance. The work, which is funded by the M.R.C. for two years, will be on amphibians and will start as soon as possible. Previous research experience in developmental biology, cell biology, or immunobiology would be an advantage. Salary in accordance with age and qualifications up to an initial maximum of £3,582 per annum. Applicants with suitable qualifications in the biological sciences should write to Dr M. J. Manning, Department of Zoology, University of Hull, HU6 7RX, before February 1, 1978, and should include a full curriculum vitae and the names of two referees. 985(A)

CENTRAL ELECTRICITY GENERATING BOARD RESEARCH DIVISION BERKELEY NUCLEAR LABORATORIES RESEARCH OFFICERS

Applications are invited from Chemistry, Engineering and Physics Graduates for vacancies at Berkeley Nuclear Laboratories.

The Laboratories are situated on the banks of the River Severn mid-way between Bristol and Gloucester. Successful candidates would be joining research teams contributing to the successful and safe operation of Board's plant both nuclear and conventional.

Salaries for these appointments will be within the following grades of the National Joint Board Agreement, according to age and experience:

Band 2 £5,160 to £6,310/£6,910
Band 3 £4,410 to £5,760
Band 4 £2,610 to £4,860

Per annum plus £120 per annum Allowance plus relevant Salary Supplements.

Applications stating age, qualifications, experience, present position and salary, should be forwarded to the Personnel Officer, Berkeley Nuclear Laboratories, Berkeley, Gloucestershire GL13 9PB not later than January 28, 1978.

Please mark envelopes "Confidential" and quote Vacancy Notice Number: BNL 33/77. 1001(A)

ULSTER MUSEUM Botanic Gardens Belfast

Applications are invited for the following posts:

(1) ASSISTANT KEEPER ZOOLOGY

The person appointed will be responsible to the Keeper of Botany and Zoology for all matters pertaining to the vertebrate collections. This will include the curation of the collections; expansion of the collections by purchase or field collecting; answering of academic and public enquiries; dealing with the 'media' on vertebrate matters; preparation of popular and academic publications and preparation of public displays in conjunction with a design team.

The person appointed will also be expected to carry out research on an aspect of vertebrates relevant to the work of the Department.

Salary:

Assistant Keeper I £4404-£7109 per annum
Assistant Keeper II £2395-£3900 per annum
Plus supplements in each case of between £443 and £521 per annum.

An honours degree in Zoology is a requirement, and appointment at Assistant Keeper I is normally dependent upon the applicant being 30 years of age and having had considerable museum experience.

(2) SENIOR GRAPHIC DESIGNER

The person appointed will hold a senior position within a team of designers involved in the structure and graphics of exhibitions and displays.

Candidates must hold a recognized graphic design qualification and have at least three years' experience in their own field of work. A knowledge of three-dimensional design is essential.

Salary:

Graphics Officer IV £2425 (age 21)—£2970 (age 27) to £3450.

Plus supplements of between £443 and £521 per annum.

Application forms for these posts may be obtained from the Administrative Officer, Ulster Museum, Botanic Gardens, Belfast BT9 5AB.

Closing date for receipt of completed application forms: Monday, 23rd January, 1978.

998(A)

CSIRO AUSTRALIA Postdoctoral Research Fellow/ Research Fellow

Division of Process Technology North Ryde, N.S.W.

CSIRO has a broad charter for research into primary and secondary industry areas. The Organization has approximately 7,000 employees—2,300 of whom are research and professional scientists—located in Divisions and Sections throughout Australia.

Field: Chemical Engineering/Fuel Technology

General: In order to accelerate the introduction of fluidized-bed combustion of coals into Australia, the Division intends to expand its research effort in this area and seeks a suitable qualified scientist who wishes to make a significant contribution.

Duties: Research will be particularly concerned with the combustion characteristics of Australian coals, the influence of inorganic constituents and the implications of these with respect to the design and operation of fluidized-bed combustors.

Qualifications: A Ph.D. or equivalent research experience in Chemical Engineering, Fuel Technology or Applied Science. Previous experience in high temperature research, especially combustion or fluidized-beds, an advantage.

Salary: Research Scientist or Senior Research Scientist: A\$14,292 to A\$20,822 p.a.

Tenure: Fixed term of three years.

Applications (in duplicate), stating **FULL** personal and professional details, the names and addresses of at least two professional referees, and quoting reference number **588/19** should reach: The Personnel Officer, Australian Scientific Liaison Office, Canberra House, Maltravers Street, LONDON WC2R 3EH by **10th February 1978**. Applications in U.S.A. and Canada should be sent to: The Counsellor (Scientific), Embassy of Australia, 1601 Massachusetts Avenue, N.W., WASHINGTON D.C. 20036 U.S.A. 995(A)

PLANT SYSTEMATIST

Applications and nominations are invited for a tenured Curator-Professorship in the Arnold Arboretum and the Gray Herbarium, Harvard University. Position involves teaching, administrative duties in connection with the combined herbaria of the Gray Herbarium and Arnold Arboretum, and research. A broadly trained herbarium-oriented systematist with interest and ability in administration and demonstrated ability in teaching and systematic research is sought for this position in a stimulating academic environment. Position effective July 1, 1979.

Letters of application or nomination with resume and three references should be sent by February 28, 1978, to Professor C. E. Wood, Harvard University Herbaria, 22 Divinity Avenue, Cambridge, Mass. 02138.

An Affirmative Action/Equal Opportunity Employer.

1012(A)

UNIVERSITY OF LONDON KING'S COLLEGE SPECTROSCOPY ASSISTANT

Applications are invited for the above Technician Grade 5 post in the Physics Research Department to work mainly for the Infra-Red and High Pressure Groups. Duties will include: Crystal growing, operation of Infra-Red and Laser Raman Spectrometers, low temperature crystals and high pressure equipment and glass blowing techniques. Initial instruction will be given if required. Minimum qualifications H.N.C. or equivalent plus relevant experience. Salary £3,651 p.a. (inclusive) rising to £4,185 p.a. (inclusive). 4 weeks annual holiday. Contributory pension scheme. Apply in writing with full details: The Head Clerk, Ref. 191106/N, King's College London, Strand WC2R 2LS.

1003(A)

EQUINE RESEARCH STATION ANIMAL HEALTH TRUST NEWMARKET

Microbiologist, preferably with postgraduate qualifications — Dip.Bact. and/or Ph.D., required to join research team investigating Contagious Equine Metritis. Initially the biology and growth characteristics of the micro aerophilic organism are to be studied and collaborative investigations undertaken to improve methods of diagnosis. Further work will involve organisms inhabiting the equine genitalia in health and disease. The appointment will be for three years with a possibility of permanency.

Salary scale in the range £6,489 to £7,509 depending upon experience and qualifications. There is some urgency in making the appointment and applications giving full address plus two referees should reach the Director, Animal Health Trust, Equine Research Station, P.O. Box 5, Balaton Lodge, Snailwell Road, Newmarket, Suffolk CB8 7DW (telephone number 0638-61111), before January 27, 1978.

957(A)

Charing Cross Hospital DEPARTMENT OF MEDICAL PHYSICS BASIC GRADE PHYSICIST

Applications are invited for the above vacancy in this teaching hospital. Applicants should have a first or second class Honours degree in Physics. They will be mainly concerned with the uses, measurement and application of radiations in the medical field. They will also be expected to participate in the development of clinical instrumentation.

Previous experience is desirable, but not essential, and need not be in the medical field.

Salary is related to qualifications and experience, and is in accordance with Whitley Council Agreements, in the range of £3,090 to £4,258 inc., or £3,540 to £4,258 inc.

Application forms and further details may be obtained from the District Personnel Department, Charing Cross Hospital, Fulham Palace Road, London W6 8RF. Tel: 01-748 2040 ext. 2997. Closing date for return of applications February 3, 1978.

972(A)

CHEMICAL ENGINEERING

Applications are invited for appointment as Assistant Professor in Chemical Engineering. (Associate rank may be considered for an outstanding candidate). Qualifications required are a Ph.D. (or equivalent) in Chemical Engineering with research or industrial experience in fluidized reactor engineering. The successful applicant will be required to teach graduate and undergraduate courses in chemical engineering, including undergraduate core courses; and to conduct research and supervise graduate students in fluidized reactor engineering. The appointment is effective July 1, 1978. Closing date for applications is April 15, 1978. Applications including curriculum vitae and names of three referees may be sent to:



G. F. Chess, P.Eng.
Acting Dean
Faculty of Engineering Science
The University of Western Ontario
London, Ontario, Canada
N6A 5B9

960(A)

THE UNIVERSITY OF TORONTO

DEPARTMENT OF BOTANY

Applications are invited for a tenure-stream position as Assistant Professor in the Department of Botany. The position will be filled either by a plant virologist or by a higher plant taxonomist; candidates from both disciplines are invited to apply. A doctoral degree will be required, with commensurate research and teaching experience. The starting date for the post will be July 1, 1978. The salary range for Assistant Professor is currently \$15,900 to \$27,100, with starting salary determined by experience.

Further particulars can be obtained from Professor T. C. Hutchinson, Chairman, Department of Botany, University of Toronto, Toronto M5S 1A1, Canada, to whom applications and the names and addresses of three referees should be sent before March 1, 1978.

1013(A)

The University of Sheffield DEPARTMENT OF LANDSCAPE ARCHITECTURE EXPERIMENTAL OFFICER

Applications are invited for the above appointment to assist with the development of project teaching in courses for landscape architects up to postgraduate and professional levels. There are also opportunities to take part in departmental research. A scientific background with a postgraduate qualification or experience of landscape design, planning, conservation or landscape management is required. Salary in the range £2,904 to £3,547 on the OR 1B scale £2,904 to £4,190, with superannuation provision. Further particulars from the Registrar and Secretary, The University, Sheffield S10 2TN to whom applications (5 copies), should be sent by March 1, 1978. Quote Ref. R.61/G.

989(A)

MEDICAL RESEARCH COUNCIL TECHNICIAN/RESEARCH OFFICER

There is a vacancy for a Technician/Research Officer to work in the genetic analysis of human lymphoblastoid cell lines with particular emphasis on their surface antigens and the production of immunoglobulins *in vitro*.

Applicants should have H.N.C. or a degree in a relevant biological subject and experience in tissue culture work would be an advantage.

Salary, dependant upon grade, would be on a scale £2,511 to £3,738 or £2,790 to £3,936 both plus pay and variable pay supplements. The post is permanent and superannuable and conditions of employment are very good.

Applications in writing and including the names, etc., of two referees to the Administrative Officer, M.R.C. Clinical and Population Cytogenetics Unit, Western General Hospital, Crewe Road, Edinburgh EH4 2XU, to arrive by January, 1978.

1021(A)

UNIVERSITY OF RHODESIA
LECTURER/
SENIOR LECTURER
in the
DEPARTMENT OF GEOLOGY

Applications are invited for appointment to Senior Lecturer/Lecturer in the Department of Geology. Applicants should have at least an honours degree in Geology and be competent to teach Sedimentology. Experience in one or more of the fields of structural, metamorphic and economic Geology would be an added recommendation.

Salary Scales (Approx. Sigs. Equiv.):
Senior Lecturer—£7,352 by 278 to £7,630 by 288 to £7,918 by 298 to £9,706; Lecturer Grade I—£6,675 by 248 to £7,667; Lecturer Grade II: £4,118 by 218 to £5,208 by 248 to £6,448.

Permanent Pensionable Terms:
Family passages and allowance towards transport of effects on appointment. Installation loan of up to half of one year's salary if required. Unfurnished University accommodation guaranteed for a period of at least three years for persons recruited from outside Rhodesia. Sabbatical Leave and Triennial visits with travel allowances. Superannuation and medical aid schemes.

Short-term Contracts: Family passages and allowance towards transport of effects. Assistance with accommodation for persons recruited from outside Rhodesia.

Applications: Six copies giving full personal particulars (including full name, place and date of birth, etc.), qualifications, experience and publications and names and addresses of three referees, should be submitted by January 31, 1978, to the Senior Assistant Registrar (Science), University of Rhodesia, P.O. Box MP 167, Mount Pleasant, Salisbury, Rhodesia. Overseas applicants should send a copy of their applications to the Association of Commonwealth Universities (Appts), 36 Gordon Square, London WC1H 0PF, from whom conditions of appointment may be obtained.

British subjects considering applying for posts in Rhodesia are urged to consult the Foreign and Commonwealth Office (telephone 233 4143) or their nearest British Consular Office. 1025(A)

Nature Conservancy Council
SCIENTIFIC OFFICER/
HIGHER SCIENTIFIC
OFFICER

Salary £2,592 to £4,032 (S.O.), £3,745 to £4,976 (H.S.O.) Starting salary may be above the minimum depending on age, qualifications and experience. Promotion prospects to £6,300 and above.

Applications are invited for the following post:—

ASSISTANT REGIONAL OFFICER—
CLWYD

The A.R.O. will be based at the County Planning Office at Mold and will represent the N.C.C. in the county of Clwyd which, with Gwynedd, comprises the North Wales Region of N.C.C. Duties of the post will include the conservation of Sites of Special Scientific Interest, provision of advice on wildlife conservation to planning authorities and other official bodies, landowners and the voluntary interests. The Officer appointed will also organise and conduct ecological surveys and liaise with the public, including lecturing and field visits and assist in the preparation of leaflets.

Candidates should normally be under 30 years of age with a degree (or equivalent) in Geology, Zoology, Botany or Geography. A current driving licence is essential.

Application forms and further particulars are available from Miss L. Bull, Recruitment Section, Nature Conservancy Council, P.O. Box 6, George Street, Huntingdon, Cambs. PE18 6BU (Tel. Huntingdon (0480) 56191 ext. 277). Please quote reference number: E2/18/17.

Closing date for completed application forms: February 3, 1978. 979(A)

ICI Central Toxicology Laboratory

Research Assistant

This Laboratory provides advisory and experimental services to ICI Ltd, for the control of toxic hazards in manufacturing processes and in other fields of growing importance, such as the development of pesticides and food additives. Emphasis is placed on improving and assessing methods for studying the biological effect of foreign compounds and upon understanding mechanisms of toxicity. The provision of toxicological information, prior to product clearance and registration in many countries, is a major activity undertaken by the Laboratory on behalf of the ICI group.

We are looking for a Research Assistant with a degree or equivalent in biochemistry. He/she will join a small applied biochemistry group whose remit is to contribute to the development of both new predictive approaches and routine procedures for use in the determination of the acute toxicological effects of ICI compounds. Specifically, the

main interest of this group lies in the assessment of primary irritancy of the skin. The job holder will have the ability to work on his/her own initiative with the flair and skill to adapt, improve and modify experimental techniques as conditions demand.

Although not essential, previous practical experience in enzyme kinetic measurements, spectrophotometry and the use of radioisotopes would be an advantage.

Requests for application forms, quoting Reference RA/AT/GO, should be addressed to:



Miss S. C. Carson
Personnel Officer—
ICI Limited
Central Toxicology Laboratory
Alderley Park
Nr. Macclesfield, Cheshire

OVERSEAS CANDIDATES SHOULD ONLY
APPLY IF THEY HAVE A PLANNED VISIT TO
THE UK WITHIN THE NEXT TWO MONTHS.

1018(A)

**THE UNIVERSITY
OF LEEDS**

**DEPARTMENT OF
MICROBIOLOGY**

Applications are invited for the post of LECTURER, available from October 1978. The successful candidate will be expected to assist in teaching bacteriology at all levels of the B.Sc. course in Microbiology. An interest in medical bacteriology (this would be taken to include those with a record of research achievement in fundamental bacteriology interested in collaborative research with clinical bacteriologists) or in bacterial physiology would be particularly welcome, but is not essential. Salary on the scale for Lecturers £3,333 to £6,655. Application forms and further particulars may be obtained from the Registrar, The University, Leeds LS2 9JT, quoting reference number 96/7D. Closing date for applications, February 3, 1978. 990(A)

**FACULTY POSITION—
PLANT BIOCHEMIST**

Applications are requested for an appointment as ASSISTANT PROFESSOR OF BIOCHEMISTRY in the tenure track at the University of California, Riverside, effective Summer 1978. Applicants should have demonstrated ability in research in plant biochemistry and the capability to develop a vigorous research program in this area. This individual will be expected to teach a course in plant biochemistry at the upper division/graduate level and participate in other teaching activities of the department. Candidates should send curriculum vitae, a statement of their current and future research interests and ask three scientists familiar with their work to send us letters of recommendation. Applications will be accepted until March 10, 1978 or until a suitable candidate is located. Address correspondence to: SEARCH COMMITTEE, PLANT BIOCHEMIST, Department of Biochemistry, University of California, Riverside, CA 92521. The University of California is an Equal Opportunity/Affirmative Action Employer. Minority and women candidates are encouraged to apply. 969(A)

**M.R.C. NEUROCHEMICAL
PHARMACOLOGY UNIT
NEUROANATOMIST**

A vacancy exists for a non-clinical scientist to carry out neuroanatomical research, with an emphasis on studies of the distribution of various neurotransmitters and drug receptors in mammalian CNS. The person appointed would be expected to develop their own lines of research in this area, and to collaborate with other members of the scientific staff. Modern light and electron microscope facilities are available, and substantial quantities of human post-mortem brain tissue can be made available for research purposes if desired.

Candidates should hold a Ph.D. in neuroanatomy or a related subject and experience with modern anatomical and histochemical techniques would be desirable. The position is available from August 1, 1978 and would be initially for a term of 5 years.

Salary depending on age and experience, within the range £4,190 (to £5,219) or £5,423 (to £6,655). M.R.C. conditions of service. The post is superannuable.

Applications in writing please to Dr L. Iversen, Director, M.R.C. Neurochemical Pharmacology Unit, Department of Pharmacology, Medical School, Hills Road, Cambridge CB2 2QD. Closing date for applications, February 17, 1978. 996(A)

AN FORAS TALUNTAIS
GRADUATE IN AGRICULTURAL SCIENCE OR
VETERINARY SCIENCE

Required for research on carcass composition with particular reference to the assessment of carcass quality.

ESSENTIAL: An Honours University degree, or equivalent, in Agricultural Science or a University degree, or equivalent, in Veterinary Science.

DESIRABLE: Suitable research experience and/or experience in Animal Husbandry.

Appointment up to and including Senior Research Officer level, depending on qualifications and experience. Non-contributory Pension Scheme with provision for Widows' and Orphans' pensions.

Application form and further particulars may be obtained from The Personnel Department, (Ref: MRD2), An Foras Taluntais, Headquarters, 19 Sandymount Ave., Ballsbridge, Dublin, 4. (Phone 688188). Latest date for receipt of completed forms Monday January 30, 1978. 962(A)

TECHNICIAN

required to join a research team investigating certain aspects of mammalian meiosis. H.N.C. or equivalent qualification required. Applications to the Administrative Assistant, Paediatric Research Unit, The Prince Philip Research Laboratories, Guy's Tower, London Bridge, SE1 9RT. 1008(A)

THE UNIVERSITY OF BRITISH COLUMBIA

In anticipation of increased activity in coal exploration, production and utilization within the Province of British Columbia, the University of British Columbia, a government funded institution which provides instruction and research opportunities to 23,000 students, is expanding its coal activities. A new coal research laboratory is scheduled for completion in 1978 and the following faculty appointments are expected to be made in August, 1978.

Within the Department of Mineral Engineering:

Coal Processing—Working in conjunction with three mineral processing faculty members, the appointee can expect to lead the coal processing activities of the department by providing appropriate instruction and research direction to undergraduate and graduate students.

Applicants are expected to be holders of advanced degrees who, through participation in coal-processing plant design and operation, and/or coal processing research, are well versed in current technology.

Mine Services—In collaboration with three mining engineer faculty members, the appointee will engage in the teaching and research aspects of mine ventilation, materials handling systems, and the control of dust and noxious gases. The appointee will be a graduate engineer who, through appropriate underground mining experience, is conversant with modern practice in the specific fields mentioned above. Coal mining experience is a decided asset for this appointment.

Rock Mechanics—The appointee will hold an appropriate advanced degree and will be oriented to the measurements of in-situ rock stress distributions and the design of underground openings in strata-bound sequences. In cooperation with three mining engineer faculty members, the appointee will be responsible for undergraduate instruction and graduate studies in applied rock mechanics. Currently the department possesses a variety of rock properties measurement equipment and has direct access to an extensive computer facility.

Within the Department of Geological Sciences:

Coal Geologists—The department is seeking a geologist who will develop a coal geology program that integrates with the coal activities in Mineral Engineering. The appointee will be involved in teaching coal geology, the supervision of graduate students, and a research program in geologic aspects concerned with mine development, such as structure, stratigraphy, correlation, petrology, and geo-statistics. A Ph.D., in hand or pending, and field experience in coal geology are essential. It is expected the appointment will be made at the Assistant or Associate level.

Applicable salary ranges are as follows:

Assistant Professor	\$20,000—\$27,000 per annum
Associate Professor	\$27,000—\$32,000 per annum
Full Professor	\$30,000—\$35,000 per annum

Enquiries, or applications accompanied by a full curriculum vitae and the names of three referees, should be directed as follows:

a) for positions within Mineral Engineering

The Head,
Department of Mineral Engineering,
University of British Columbia,
VANCOUVER, B. C., Canada V6T 1W5

b) for the position within Geological Sciences

Dr. W. H. Matthews
Chairman, Coal Committee
Department of Geological Sciences,
University of British Columbia,
VANCOUVER, B. C., Canada

This announcement should not be construed as a commitment by U.B.C. to make appointments in rigid adherence to the above descriptions. 926(A)

Rothamsted Experimental Station

Harpden, Herts. AL5 2JQ ASSISTANT MANAGER (Male or Female)

required for mainly arable 800 acre farm. Duties will be mainly concerned with the provision of technical information and services to field staff working on farm and field experiments. The successful applicant will be required to assist with office and farms organisation and to liaise with scientific staff.

Qualifications: Pass degree (Agric.), H.N.C., or equivalent with at least 5 years' relevant post qualifying experience.

Appointment in grade of Higher Scientific Officer, salary range £3,776 to £4,976 including current pay supplements. Non-contributory superannuation.

House available to rent.

Apply in writing to the Secretary giving names and addresses of two referees and quoting ref. 344 by January 3, 1978. Further details on request. 980(A)

ECOLOGIST-ASSOCIATE DIRECTOR, U.C.L.A.

Applications are invited from highly qualified ecologists to become Chief of Division of Environmental Biology and Associate Director of the Laboratory of Nuclear Medicine and Radiation Biology. The position carries a joint appointment, presumably at the full professor level, in the Department of Biology, and undergraduate and graduate teaching responsibilities. Research interests should be in quantitative ecology and applicants should be interested in resolving environmental problems related to energy technologies because the Laboratory's research is funded primarily by the Department of Energy (formerly E.R.D.A.).

Send letters of inquiry or application, including curriculum vitae, description of research and teaching interests, and names and addresses of three referees before February 15, 1978 to Director's Office, Laboratory of Nuclear Medicine and Radiation Biology, University of California, 900 Veteran Avenue, Los Angeles, California, 90024. An equal opportunity/affirmative action employer. 902(A)

AN FORAS TALUNTAIS

GRADUATE IN CHEMISTRY OR BIO-CHEMISTRY

Required by An Foras Taluntais in its Cereal Technology Department, Kinsealy Research Centre, Malahide, Co. Dublin. The appointee will be engaged in a research programme on cereal technology and will work in close liaison with the milling and baking industries.

ESSENTIAL: Honours University degree, or equivalent, majoring in Chemistry or Biochemistry.

DESIRABLE: Suitable experience in the cereal industries.

Commencing salary and level of appointment will depend on qualifications and experience. Application forms and further particulars from the Personnel Department, Headquarters, 19 Sandymount Ave., Dublin, 4. (Tel: 01-688188). Latest date for receipt of completed application forms Friday January 27, 1978. 964(A)

UNIVERSITY OF OXFORD

Microbiology Unit DEPARTMENT OF BIOCHEMISTRY

POSTDOCTORAL RESEARCH ASSISTANT— Ref. B14

There is a vacancy for a post-doctoral biochemist or microbiologist to work on bacterial sporulation. The appointment, which will be for one year in the first instance, on the scale £3,333 to £5,627, may be extended for a further period of two years and there is provision for U.S.S. membership.

Applications, which should include relevant details and the names of two referees, should be sent before February 28, 1978 to Professor J. Mandelstam, Microbiology Unit, Dept. of Biochemistry, South Parks Road, Oxford OX1 3QU.

DEPARTMENTAL DEMONSTRATORSHIP— Ref. B15

It is proposed to appoint a Departmental Demonstrator from October 1, 1978. The salary will be on the scale £3,333 to £3,833, according to qualifications and experience and with membership of U.S.S. The appointment is for a period not exceeding 3 years. The successful candidate will be expected to take part in departmental teaching and to conduct his or her own research.

Applications (3 copies) including the names of two referees, should be sent to: The Administrator, Department of Biochemistry, South Parks Road, Oxford OX1 3QU not later than February 28, 1978. 863(A)

New Zealand UNIVERSITY OF CANTERBURY Christchurch

Applications are invited for the following positions:

LECTURER OR SENIOR LECTURER IN CIVIL ENGINEERING

Applicants should have a good degree and a specialised knowledge in the history of civil engineering. An interest in structural mechanics or engineering materials would also be an advantage. Applications close on April 30, 1978.

LECTURER IN ZOOLOGY

Applicants should be qualified to teach undergraduate genetics and will be expected to lecture and conduct laboratory classes in this branch of zoology. Applications close on February 28, 1978.

The salary for Senior Lecturers is on a scale from NZ\$13,424 to \$15,411 (bar), \$16,546 per annum and for Lecturers is on a scale from NZ\$10,585 to \$12,969 per annum.

Particulars, including information on travel and removal allowances, study leave, housing and superannuation, may be obtained from the Association of Commonwealth Universities (Appts), 36 Gordon Square, London WC1H 0PF. 1026(A)

UNIVERSITY OF YANNINA

SCHOOL OF MEDICINE

"VACANT CHAIRS IN GENERAL BIOLOGY AND BIOCHEMISTRY"

The School of Medicine at the University of Yannina would like to inform that shortly they will be advertising in the Government's Official Gazette two vacant chairs, one in General Biology and one in Biochemistry.

For further information, concerning details and the exact date of publication of the vacancies in the Government's Official Gazette, the applicants should contact the Secretariat of the School of Medicine, University of Yannina.

Tel: 0651 21802 Monday-Saturday between 8.00 a.m.-2.00 p.m. 935(A)

THE OPEN UNIVERSITY

RESEARCH FELLOW IN EARTH SCIENCES

The Research Fellow will assist Dr P. W. Francis in continuing studies of recent volcanism in the Central Andes. The work will be concerned with studies of large volume ignimbrites and their source structures and other volcanic problems, and will involve extensive field work. Applicants should have a Ph.D. and research experience in volcanology, preferably on recent pyroclastic flows. They should also have experience of working in remote areas. Knowledge of Spanish is an advantage. The Fellowship is funded by the N.E.R.C. and is for a period of three years commencing as soon as possible, after January 1, 1978.

The salary will be in the range £3,333 to £3,761 p.a. Further particulars and application forms are available from the Personnel Manager (RFE2) P.O. Box 75, The Open University, Walton Hall, Milton Keynes, MK7 6AL. Telephone: Milton Keynes 63868. Closing date for applications January 26, 1978. 1005(A)

CHEMISTRY LIBRARIAN

LIBRARIAN II or III

Available July 1, 1978. Responsible for the management and operation of the Chemistry/Biochemistry Library. Advanced degree or relevant experience in the subject field and M.L.S. preferred; relevant library experience desirable. Salary in a range having base of \$13,000 (L-II) or \$15,100 (L-III) but without a ceiling. Send résumé, three letters of recommendation and transcripts, by February 15, 1978, to:

Chemistry Librarian Search Committee,
c/o Maria G. Larson
Princeton University Library
Princeton, N.J. 08540.
Equal opportunity/affirmative action employer. 927(A)

UNIVERSITY OF THE WEST INDIES JAMAICA

Applications are invited for the following posts:

LECTURER/ ASSISTANT LECTURER in the DEPARTMENT OF BIOCHEMISTRY

Duties of the appointee will include lecturing and demonstrating to classes of undergraduates in both the Medical and Natural Sciences Faculties. Preference will be given to candidates with medical qualifications. The appointment will run until September 30, 1979.

Salary scales: Senior Lecturer J\$15,339 to J\$19,326 p.a.; Lecturer (Medical) J\$11,262 to J\$15,015 p.a.; (Non-Medical) J\$8,913 to J\$13,917 p.a.; Assistant Lecturer (Medical) J\$9,102 to J\$9,936 p.a.; (Non-Medical) J\$7,236 to J\$8,412 p.a. (£1 sterling=J\$2.34.) F.S.S.U. Study and Travel Grant. Unfurnished accommodation will be let by the University at a rental of 10% of full passages (at approved rates) on appointment and on normal termination.

Detailed applications (three copies) giving full particulars of qualifications and experience, date of birth, marital status and the names and addresses of three referees should be sent as soon as possible to the Registrar, University of the West Indies, Mona, Kingston 7, Jamaica. Further particulars of the post are available from the same source. 1017(A)

MEMORIAL UNIVERSITY OF NEWFOUNDLAND

Pharmacologists with a Ph.D. and/or M.D. degree are invited to apply for a faculty position at the Faculty of Medicine, Memorial University of Newfoundland. The duties will include contributing to the teaching of pharmacology in an integrated medical curriculum as well as some pharmacology teaching to science students and to graduate students. Candidates should have a broad background in general pharmacology with a major interest in drug kinetics, toxicology, drug metabolism or biochemical pharmacology and will be expected to have or to develop a research program. The Faculty of Medicine has recently moved into a new Health Sciences Complex located on the Memorial University Campus, and will soon be joined by the St. John's General Hospital.

Academic rank and salary will be commensurate with experience and qualifications. The position will be available from September 1978 or by negotiation. The application deadline is March 10, 1978. Application, including curriculum vitae and the names and addresses of three references, should be sent to: Dr R. S. Neuman; Chairman, Pharmacology Search Committee; Faculty of Medicine, St John's, Newfoundland, Canada A1B 3V6. 812(A)

HARVARD UNIVERSITY

seeks a biological anthropologist with a specialisation in paleoanthropology and human evolution, as well as a specialisation in human anatomy. This is for a permanent position as a full professor beginning in September 1978. The appointment is to be made jointly by the Department of Anthropology and the Department of Anatomy in the Harvard Medical School. Applicants must have a Ph.D. as well as teaching and research interests in these fields.

Kindly send curriculum vitae, references and publications to Ms Mary Clare Gubbins, Peabody Museum, 54A, Harvard University, Cambridge, Massachusetts 02138. Harvard University is an equal opportunity, affirmative action employer. 925(A)

BRITISH PHARMACOPOEIA COMMISSION

PHARMACIST

... to help maintain medicinal standards

The British Pharmacopoeia Commission is responsible for all published standards for articles used in both human and veterinary medicine. The successful candidate, based at the Commission's new laboratories at Canons Park, Middx, will be concerned with the physical pharmaceutical aspects of standards for the quality control of medicinal substances and preparations. The work will centre around investigating the formulation of official preparations (including laboratory manufacture of batches of material to evaluate their stability). It will also involve devising test methods for existing and new formulations and monographs, and preparing reports for technical committees.

For further details of the work, phone Mrs S Richens on 01-952 2311 ext 397 or 395.

Candidates (normally aged under 30) should have a degree or equivalent in Pharmacy and have had experience in pharmaceutical research, product formulation or quality control.

Appointment will be as Higher Scientific Officer (£4,030 to £5,250). Starting salary according to qualifications and experience. There is a non-contributory pension scheme.

For further details and an application form (to be returned by 3 February 1978), write to Civil Service Commission, Alencon Link, Basingstoke, Hants, RG21 1JB, or telephone Basingstoke (0256) 68551 (answering service operates outside office hours). Please quote reference SB/10/KD. 1002(A)

UNIVERSITY OF NEWCASTLE UPON TYNE DEPARTMENT OF BIOCHEMISTRY RESEARCH ASSOCIATE

Applications are invited from post-doctoral biochemists, molecular biologists or microbial geneticists to join a small group investigating DNA repair and genetic recombination in *Escherichia coli*. The project is supported by a grant from the Medical Research Council and the appointment will be for three years at a salary within the scale £3,333 to £5,627 (under review).

Enquiries and applications, which should include a curriculum vitae and the names of two referees, should be sent to Dr P. T. Emmerson, Department of Biochemistry, University of Newcastle upon Tyne, Newcastle upon Tyne NE1 7RU (telephone 0632 28511, ext. 3434). 1015(A)

DEPARTMENT OF PHYSICS ASSISTANT PROFESSORSHIP

Subject to adequate funding, a position may become available July 1, 1978 in one of the following fields

QUANTUM OPTICS BIOPHYSICS

Salary depends on qualifications. The closing date for applications is March 31, 1978.

Exceptional candidates in other fields may be considered.

All correspondence should be addressed to:

Professor Luis de Sobrino,
Chairman,
Appointments Committee,
Department of Physics,
The University of British
Columbia,
2075 Westbrook Place,
Vancouver, British Columbia,
Canada V6T 1W5. 823(A)

THE DISTILLERS COMPANY LIMITED FERMENTATION BIOCHEMIST

The Yeast and Food Division of the Distillers Company Limited requires a graduate to work in their small scale Pilot Plant at Glenochil Technical Centre, Menstrie, Clackmannanshire. Studies carried out in this Pilot Plant include yeast strain selection work, process development and examination of raw materials.

Applicants, who should preferably be under 28 years old, must have a degree in microbiology, biochemistry or closely related subjects together with relevant postgraduate experience. One or two years commercial or research experience with yeast would be an advantage.

Further details together with an application form can be obtained by writing to:

The Yeast Research Manager,
Yeast and Food Division,
The Distillers Company Limited,
Glenochil Technical Centre,
MENSTRIE,
Clackmannanshire FK11 7ES.
1016(A)

ZOOLOGICAL SOCIETY OF LONDON

requires

RECORDERS FOR ZOOLOGICAL RECORD

a bibliography of the world's zoological literature.

For one vacancy in the Unit at Boston Spa, West Yorkshire, the ability to read and summarise Russian—preferably at degree level—is required. For a further vacancy which may be either at Boston Spa or in the Unit based at the British Museum (Natural History) in London, linguistic ability—while helpful—is not essential. For this post applicants should preferably possess a degree in Zoology or an allied subject.

Salary £2,737.50 on scale to £3,978.45 (including supplements). An additional allowance is payable in respect of the post to read Russian.

Application in writing, giving brief details of career to date and stating which vacancy is being applied for, should be made to the Managing Recorder, 44 High Street, Boston Spa, Wetherby, West Yorkshire LS23 6EA, by January 20. 1019(A)

QUEEN ELIZABETH COLLEGE

(University of London)
Campden Hill Road,
Kensington, London W8 7AH
TEMPORARY LECTURER
in Social Nutrition

Applications are invited for a Lectureship in Social Nutrition in the Food Science and Nutrition Department, to start October 1, 1978 or earlier and to last three years. The person appointed will be expected to organise and take some part in the teaching of courses dealing with the social, cultural and traditional factors that influence food choice, and, in addition, take part in the general teaching of nutrition.

Salary scale currently £3,355 to £6,655 plus £450 p.a. London allowance.

Further particulars and application forms may be obtained from the College Secretary. Closing date February 28, 1978. 991(A)

Director —Institute of Oceanographic Sciences

The Natural Environment Research Council (NERC) invites applications for the post of Director of the Institute of Oceanographic Sciences, which will shortly become vacant. The post is based at Godalming, Surrey.

The Institute in its present form was created by the Council in 1973 by bringing together under a single Directorate three previously separate NERC marine research establishments. These were the National Institute of Oceanography at Wormley, the Institute of Coastal Oceanography and Tides at Bidston (Birkenhead) and the Unit of Coastal Sedimentation at Taunton.

The work of the Institute covers the deep oceans, shelf seas and coastal and estuarine waters. Its research programme includes studies of ocean dynamics, currents, waves and tides; the geology of the sea floor; sedimentology; chemistry; and the biology of the deep oceans. To carry out its work at sea the Institute uses the vessels of the NERC research vessel fleet, especially RRS DISCOVERY.

An important function of the Institute is the development of scientific equipment and instrumentation for marine research, and the Institute has well-equipped engineering and electronic laboratories.

The annual expenditure of the Institute is at present £4.75m and receipts, mainly from research contracts undertaken for Government Departments and other organisations, are £2.5m.

The post calls for an outstanding marine scientist who can provide leadership in both the fundamental and applied aspects of the subject. The successful candidate must also have had experience in administration of research. The post carries the rank of Chief Scientific Officer (A) with a salary of £12,000 plus £208.80 Phase II pay supplement. There is a non-contributory superannuation scheme.

For application forms, please write to:

J. Hansford
Natural Environment Research Council
Falcon House, Fleming Way, Swindon SN1 2NG
quoting reference E2/B1/39. Closing date: 31 January 1978.

974(A)

**Natural Environment
Research Council**

British Trust for Ornithology APPOINTMENT OF DIRECTOR

The British Trust for Ornithology is seeking a well qualified ornithologist or zoologist to promote the development of the Trust as a national centre for ornithology. The director will have primary responsibility for leading the scientific work of the Trust. The appointment will be made in the Civil Service P.S.O. Grade (£6,034 to £7,725).

Further particulars may be obtained from The Administrator, British Trust for Ornithology, Beech Grove, Tring, Herts. Closing date for applications will be January 31 1978. 997(A)

UNIVERSITY OF LEICESTER RESEARCH ASSOCIATE IN CHEMISTRY

An S.R.C. Postdoctoral appointment is available for a suitable candidate to study 'The Aqueous Chemistry of Alcohols and Sugars using N.M.R. and Infra-red Spectroscopy.'

The work is a combined study with Professor M. C. R. Symons and Dr W. Derbyshire of Nottingham University, and the initial salary will be £3,333 a year, starting as soon as possible.

For details, please contact Professor M. C. R. Symons, Department of Chemistry, The University, Leicester LE1 7RH. 1006(A)

AN FORAS TALUNTAIS GRADUATE IN CHEMICAL OR MECHANICAL ENGINEERING

Required for research on the bio-engineering problems associated with the handling, chilling, freezing and preservation of meat and meat products.

ESSENTIAL: An Honours University degree, or equivalent, in Chemical or Mechanical engineering.

DESIRABLE: Suitable experience, preferably in the food industry.

Appointment up to and including Senior Research Officer level, depending on qualifications and experience. Non-contributory Pension Scheme with provision for Widows' and Orphans' pensions.

Application form and further particulars may be obtained from The Personnel Department, (Ref: MRD3), An Foras Taluntais, Headquarters, 19 Sandymount Ave., Ballsbridge, Dublin, 4. (Phone 688188). Latest date for receipt of completed forms Monday January 23, 1978. 961(A)

M.R.C. CLINICAL RESEARCH CENTRE

(Northwick Park Hospital)

Watford Road, Harrow, Middlesex, HA1 3UJ

The division of immunological medicine (Head: G. L. Asherson) has three-year appointment for IMAGINATIVE SCIENTIST with biochemical or biological interests to study T suppressor cells and their assay in animals and man. Candidates may visit the department by telephoning G. L. Asherson, 01-864 5311, ext. 2692.

Applicants must have three years postgraduate experience.

Salary within the range £3,355 to £5,219 plus £450 London allowance.

Further details and application form obtainable from Mrs J. Tucker-Bull quoting ref. 118/1/4194. Closing date: January 31, 1978. 1020(A)

INSTITUTE OF TERRESTRIAL ECOLOGY A SCIENTIFIC OFFICER

is required at the Institute's Research Station at Bush Estate near Edinburgh to join a group studying the taxonomy of bryophytes. A sound botanical training with interest in taxonomy and preferably some knowledge of bryophytes is required. The curation of a small bryophyte herbarium is an essential part of the job.

Qualifications: A degree or equivalent in Botany or in an appropriate subject.

Salary and conditions of service: Appointment will be in the grade of Scientific Officer. The salary will be in the scale £2,149 to £3,527 per annum plus Phase I pay supplement of £313.20 per annum and 5% Phase II supplement of between £130.50 per annum and £208.80 per annum. Starting pay will be according to age, qualifications and experience. Non-contributory superannuation scheme.

The Natural Environment Research Council is not a Government Department but conditions of service are similar to those of the Civil Service.

Application forms and further details are available from:

Establishments Section
Institute of Terrestrial Ecology
68 Hills Road
Cambridge CB2 1LA
Telephone: (0223) 69745

Please quote reference Bush 133. Closing date for completed applications is January 30, 1978.

**NATURAL ENVIRONMENT
RESEARCH COUNCIL**
1028(A)

UNIVERSITY OF EXETER SOUTH-WEST ENERGY GROUP PHYSICIST/ENGINEER

Devon and Cornwall County Councils and the States of Jersey have founded an Energy Group in conjunction with the University of Exeter, Plymouth Polytechnic and the Camborne Royal School of Mines, charged with the study of energy matters relating to the South-West.

The Group wishes to appoint a Physicist/Engineer to undertake work in the solar energy field in the Department of Physics. He/she will be the second of a small interdisciplinary team, working at the University, whose output will be fed back to the local authorities as part of what is hoped will eventually become a long-term project. There will be liaison with other groups in the area and it is hoped that the project will be a continuing one.

The appointment will be for up to two years in the first instance starting on April 1, 1978. The salary at appointment will be dependent upon qualifications and experience within the range £3,333 to £4,607 p.a. subject to review. The post is superannuated. Applicants should hold at least a good honours degree.

Applications (five copies) together with the names of three referees, should be sent to Miss Doreen Watson, Administrative Assistant (Appointments), University of Exeter, Northcote House, Exeter EX4 4QJ, by January 31, 1978. 1009(A)

The University of Sheffield DEPARTMENT OF CHEMISTRY POSTDOCTORAL ASSISTANT

Applications are invited for the post of postdoctoral Assistant in an investigation of the synthesis of model enzyme systems related to chorismate synthetase. Tenable up to two years commencing as soon as possible and supported by S.R.C. Salary in the range £3,333 to £3,547 on Range IA with superannuation. Applications with the names of 2 referees should be made to Dr E. Haslam, Department of Chemistry, University of Sheffield, Sheffield S3 7HF. Quote Ref. R.60/G. 988(A)

UNIVERSITY OF ULM, WEST GERMANY LABORATORY OF IMMUNOLOGY, DEPARTMENT OF MICROBIOLOGY POSTDOCTORAL RESEARCH ASSISTANT IMMUNOCHEMISTRY/ BIOCHEMISTRY

Immunologists are invited to apply for a postdoctoral research assistantship to work on a project concerned with cell interactions in the immune response with special reference to humoral mediators. The position is at least for three years, will be open January 1, 1978 or later and has a salary according to qualification and experience (£12,900 to £13,600). Candidates should preferably have a Ph.D., a background in immunochimistry is essential. Applications, including curriculum vitae, a description of research experience, relevant reprints and the names and addresses of two referees should be sent to Dr H.-D. Flad, Laboratory of Immunology, Department of Microbiology, University of Ulm, P.O. Box 4066, D-7900 Ulm, West Germany. 868(A).

ROYAL FREE HOSPITAL SCHOOL OF MEDICINE (University of London) DEPARTMENT OF BIOCHEMISTRY AND CHEMISTRY A TECHNICIAN

is required with experience of physical and chemical techniques to work with a team studying biomembrane structure and function. Some experience in electronics would be useful.

Salary on Whitley Council Scale. Application forms which are available from the School Secretary Royal Free Hospital School of Medicine, 8 Hunter Street London WC1N 1BF (telephone 01-837 5385, Ext. 8), should be returned by Friday, January 20, 1978. 994(A)

UNIVERSITY OF RHODESIA

Faculty of Science

DEPARTMENT OF BIOCHEMISTRY

Applications are invited for the following post within this Department:

CHIEF TECHNICIAN

The successful candidate will be responsible to the Head of Department for the department's technical services, equipment and staff. Prospective candidates must have one of the following qualifications or an approved equivalent and have had a minimum of five years relevant postqualification experience: (a) Diploma in Medical Laboratory Technology at Final Level, preferably in Clinical Chemistry; (b) City and Guilds Advanced Certificate in Biochemistry or Chemistry. In addition relevant experience in instrumentation, maintenance and electronics and proven administrative ability would be further recommendations.

Salary Scale (Approx. Sig. Equiv.): £4,719 by £74 to £6,911 or £7,158, depending on qualifications.

Pension and Medical Aid Schemes. Attractive Leave conditions. Family fares and allowance for transport of effects are available on appointment.

Applications: Six copies giving full personal particulars (including full name, place and date of birth, etc.), qualifications, experience and the names and addresses of two referees, should be submitted to the Assistant Registrar, Faculty of Science, University of Rhodesia, P.O. Box MP 167, Mount Pleasant, Salisbury, from whom further particulars may be obtained. Applications should reach him by not later than **January 31, 1978**.

British subjects considering applying for posts in Rhodesia are urged to consult the Foreign and Commonwealth Office (telephone 233 4143) or their nearest British Consular Office.

1024(A)

SWINBURNE COLLEGE OF TECHNOLOGY

(Melbourne, Australia)

LECTURER IN BIOPHYSICS

(Contract Appointment)

Applications are invited from graduates with a relevant higher degree in physiology, clinical biophysics or biomedical engineering and with clinical and/or research experience in cardiovascular physiology.

Teaching experience will also be considered in making the appointment.

The appointment will be by contract for three years duration including removal and repatriation expenses. Permanent appointment, during or at the expiration of the period, may be negotiated by mutual agreement.

The successful applicant will be involved in the undergraduate and postgraduate biophysics teaching programme with particular responsibility for the cardiovascular component of these courses. The successful applicant will also be encouraged to participate in an area of relevant applied research.

Swinburne College of Technology is a tertiary educational institution affiliated with the Victoria Institute of Colleges. The Department of Physics is a member department of the Faculty of Applied Science, and has responsibility for undergraduate courses in biophysics and instrumental science together with postgraduate programme in biophysics, biomedical instrumentation and instrumental science.

Salary: Lecturer II \$A14,631 to \$A16,748; Lecturer I \$A17,145 to \$A19,261 p.a.

Applications close February 18, 1978.

Further information about the position, conditions of employment and application procedure may be obtained from the Association of Commonwealth Universities (Apts), 36 Gordon Square, London WC1H 0PF.

983(A)

IMPERIAL CANCER RESEARCH FUND

Bursaries for Training in Research

A small number of Bursaries will be awarded at the laboratories in Lincoln's Inn Fields for full-time studies leading to higher Degrees in research fields relevant to cancer, that include chemistry, cell and molecular biology. The awards will be tenable for three years from October 1, 1978 with a non-supernannable grant of £2,640 a year (subject to tax) and, in some cases, additional allowances.

Applications are invited from British subjects, normally resident in the U.K. and not over 25 years of age, who hope to obtain First or Upper Second class Honours Degrees in Science in 1978.

Further details and application forms are available from:

The Personnel Officer
Imperial Cancer Research Fund
Lincoln's Inn Fields
London WC2A 3PX

Completed applications should reach the Director of Research at the above address not later than February 14, 1978.

904(A)

UNIVERSITY OF AUCKLAND

(New Zealand)

Applications are invited for the following full-time position. Conditions of Appointment and Method of Application are available from the Association of Commonwealth Universities (Apts), 36 Gordon Square, London WC1H 0PF, or from the Assistant Registrar (Academic Appointments) at the University of Auckland. Applications will be accepted at any time up to **February 28, 1978**. Candidates should note that at present all salaries are supplemented by a cost-of-living allowance of NZ\$365 per annum.

GEOGRAPHY—

PROFESSOR AND HEAD OF DEPARTMENT

The vacancy arises because of the retirement of Professor K. B. Cumberland in May 1978.

Besides being experienced in University teaching the applicants should be active in research and have a substantial record of research publications. Candidates suitably qualified in any branch of Geography will be considered.

At present professorial salaries are established within the range NZ\$19,748 to \$25,233 per annum having regard to the qualifications of the candidate concerned.

1027(A)

MEMORIAL UNIVERSITY OF NEWFOUNDLAND

DEPARTMENT OF GEOLOGY

The Department of Geology invites applications for three faculty positions in *Sedimentology* and *Marine Geology* with special reference to the Labrador continental margin (subject to the availability of funds).

One appointment will be a regular faculty position at a salary and rank commensurate with qualifications and experience. The appointee will direct and participate in the activities of a research group working in the following specialties: Mesozoic and Cenozoic clastic sedimentology, clay mineralogy, sedimentary geochemistry and micropaleontology. Applications from suitably qualified candidates with working experience in the petroleum industry will be especially welcome.

Two appointments will be at the rank of Assistant Professor (Research) and will be for a two-year term in the first instance. Applicants should have proven research ability in one (or more) of the specialist fields listed above.

Applications for all three positions, which must include a detailed curriculum vitae and the names and addresses of three referees, should be sent by March 31, 1978, to: Dr David Skevington, Head, Department of Geology, Memorial University of Newfoundland, St John's, Newfoundland, Canada A1B 3X5.

1010(A)

Toxicologists

Continued site development has created openings for two experienced graduates to join our team evaluating the safety of novel therapeutic compounds and thereby assist in the development of new drugs.

In addition to a degree in an appropriate biological science, such as Pharmacology or Physiology, applicants should possess not less than two years' relevant practical experience. In return we offer a competitive salary and the fringe benefits associated with a major company. The Medical Research Centre is pleasantly located close to both London and Cambridge and unfurnished Development Corporation housing may be available at an economic rent.

Application forms (only) may be obtained by telephoning Harlow (0279) 419373. If you prefer, you may write, outlining your career-to-date, to:

John Atkinson, Site Personnel Manager, Beecham Pharmaceuticals, Medicinal Research Centre, The Pinnacles, Harlow, Essex, CM19 5AD.

Please quote reference: 9/N

977(A)

Beecham Pharmaceuticals

TECHNICIAN

with training in electron microscopy to join a laboratory working on biogenesis of membrane proteins. The job involves light and electron microscopy, autoradiography, immunocytochemistry and freeze etching. The post may be renewable for up to 3 years. Salary according to age and experience but not less than \$12,000 per annum.

Applications including curriculum vitae and addresses of at least two referees to Dr J. J. Kraehenbuhl, Institut de Biochimie, University of Lausanne, 1066 Epalinges, Switzerland.

958(A)

Institute of Cancer Research

TECHNICIAN

required to work on the mechanism of action of cytotoxic and carcinogenic compounds at Pollards Wood Research Station, Chalfont St. Giles, Bucks. H.N.C. or degree qualification required. Knowledge of sterile techniques and radioisotope labelling an advantage. Starting salary dependent on age and experience, not less than £2,964 p.a.

Applications in duplicate with the names of two referees to the Secretary, Institute of Cancer Research, 34 Sumner Place, London SW7 3NU quoting ref. 301/B/37.

981(A)

Ministry of Agriculture, Fisheries & Food, Pest Infestation Control Laboratory, Tolworth, Surrey.

Organic Chemist

■ Work on molecular basis of rodenticide action and resistance to anticoagulants
■ Synthesise novel organic chemical compounds
■ Study their interaction with biological systems in vivo/vitro.

□ Good honours degree or equivalent in appropriate scientific subject; final year students will not be considered
□ Experience of organic chemical synthesis essential
□ Interest in biological action of xenobiotics and a working knowledge of modern chromatographic techniques desirable
□ Age normally under 27
□ Appointment as Scientific Officer (£2870 - £4320) □ Ref: SB/34/AF.

■ Application forms (for return by 27 January 1978), from Civil Service Commission, Alencor Link, Basingstoke, Hants, RG21 1JB, telephone Basingstoke (0256) 68551 (answering service operates outside office hours).

1007(A)

Science Group
CIVIL SERVICE

free university amsterdam

The Department of Physics and Astrophysics has a vacancy for a

professor in (experimental) physics

An experimental physicist is required with experience in the field of intermediate energy physics and with interest in its theoretical aspects.

The person to be appointed is expected to cooperate with the present scientific staff of the Experimental Nuclear Physics Section in leading the current research of the laboratory, concentrated very much around the available AVF-cyclotron (maximal proton energy 30 MeV). He is, in particular, expected to contribute to a development of research in intermediate energy physics of this group to be conducted with the 500 MeV linear electron accelerator under construction at the I.K.O., Amsterdam.

To start a group of his own 2 or 3 positions are available. A gradual transition from the current job to the Free University can be arranged.

In due course he will have to take part in the education provided by the Department of Physics and Astrophysics, which will involve both lectures and the supervision of students undertaking research projects in the section.

The University hopes to find a candidate who can agree with the Christian aim and objective of the University.

Interested persons or those wishing to recommend another for the post should contact the secretary of the appointing committee, Prof. dr. H. Verheul, Natuurkundig Laboratorium der Vrije Universiteit, De Boelelaan 1081, Amsterdam - Buitenveldert, Phone: 020-584 24 69.

Applicants are requested to submit a curriculum vitae and a list of publications together with their application to Dienst Personeelszaken, De Boelelaan 1105, Post Office Box 7161, Amsterdam, not later than March 1, 1978 mentioning vacancy number 311-3045.

959(A)



M.R.C. NEUROCHEMICAL PHARMACOLOGY UNIT PEPTIDE CHEMIST

A temporary scientific staff position is available to develop work on the synthesis of analogues of peptides known to exist and act within the central nervous system, including the enkephalins and substance P. The person appointed will work closely with Unit staff involved in the assessment of the biological activity of such peptide analogues, and the chemical work will be undertaken with the supervision and guidance of Dr R. Shepard in the M.R.C. Laboratory of Molecular Biology nearby.

Candidates should ideally be aged 24-27 and have some previous training in peptide chemistry or a related subject to Ph.D. level or equivalent. The post is available for 3 years from April 1, 1978 or earlier.

Salary depending on age and experience in scale £3,975 to £5,219 M.R.C. conditions of service, including superannuation.

Applications in writing please to Dr L. Iversen, Director, M.R.C. Neurochemical Pharmacology Unit, Department of Pharmacology, Medical School, Hills Road, Cambridge CB2 2QD. Closing date for applications, February 17, 1978.

993(A)

TECHNICIAN

required in the Standards Laboratory for Serological Reagents. Suitable qualifications would include a relevant science degree, A.I.M.L.S., or H.N.C. in Medical Laboratory Sciences with experience in microbiology. The vacancy occurs in the unit concerned with the development and production of viral diagnostic reagents.

Salary according to age and experience, but not less than £2,184 per annum plus £354 London Weighting plus £454.50 Pay Supplements 1 and 2.

Applications with the name of at least two referees to Personnel Officer, Central Public Health Laboratory, Colindale Avenue, London NW9 5HT. Tel. 205 7041. 966(A)

LOUGHBOROUGH UNIVERSITY OF TECHNOLOGY

Radiochemistry Research

Applications are invited for the post of S.R.C. RESEARCH ASSISTANT to participate in a project on radiolabelling drug molecules. Experience in radiochemistry or in biochemistry is desirable but not essential.

The appointment is for two years at a starting salary of £3,333 (under review). Application forms and further details from Dr David Malcolm-Lawes, Department of Chemistry, Loughborough Leicestershire 1014(A)

AN FORAS TALUNTAIS GRADUATE — MEAT INDUSTRY LIAISON OFFICER

AN FORAS TALUNTAIS invites applications for the above post in its Meat Research Department at Dunsinea Research Centre, Castleknock, Co. Dublin. The appointee will maintain technical liaison with the meat industry and will provide an information service on current research and development in meat science and technology.

ESSENTIAL: He/she will have an Honours University degree, or equivalent, as well as a food science or engineering background.

Experience in the food industry (preferably in the meat processing sector) will be an advantage. Commencing salary and level of appointment will depend on qualifications and experience. Non-contributory Pension Scheme with provision for Widows' and Orphans' pensions.

Application forms and further particulars from The Personnel Department, (Ref: MRD1), An Foras Taluntais, Headquarters, 19 Sandymount Ave., Dublin, 4. (Phone 01-688188). Latest date for receipt of completed forms Friday January 27, 1978. 963(A)

LINCOLN COLLEGE (University College of Agriculture) New Zealand LECTURER IN BIOCHEMISTRY

The Council of Lincoln College invites applications for the position of Lecturer in Biochemistry.

Applicants should have a degree in Science or Agricultural Science, and a postgraduate qualification. Preference will be given to those with experience in Agricultural Biochemistry. Duties will include teaching Biochemistry in the course for Bachelor of Agricultural Science, and the appointee will be expected to carry out research in Biochemistry related to Agriculture.

Commencing salary according to qualifications and experience within the range NZ\$10,239 to NZ\$12,543 per annum. At present salaries are supplemented by an "Interim Special Allowance" of 3.5%.

Travel and removal expenses reimbursed up to specified limits. New Zealand Government Superannuation available.

Conditions of Appointment are obtainable from the Association of Commonwealth Universities (Appts), 36 Gordon Square, London WC1H 0PF, or from the Registrar of the College.

Applications close March 6, 1978. 982(A)

FACULTY OPENING IN BIOCHEMISTRY University of Illinois Champaign-Urbana

The Department of Biochemistry is seeking applicants for a tenure track appointment at the level of Assistant Professor or higher to begin August, 1978. Applicants should be qualified to teach biochemistry courses at undergraduate or graduate levels. Only candidates who have demonstrated excellence in research and who are prepared to establish an active research program will be considered, preference will be given to those who have received graduate and postdoctoral training in strong research environments. The minimum salary at the Assistant Professor level is \$15,000 for a nine-month appointment; salaries at higher ranks are negotiable. For full consideration, application materials, including a curriculum vitae, a list of publications, a brief description of research interests and three letters of reference should be sent by February 1, 1978 to:

Professor Lowell P. Hager, Head
Department of Biochemistry
University of Illinois
Urbana, Illinois 61801

Telephone: (277) 333-3945.
The University of Illinois is an Affirmative Action/Equal Opportunity Employer. 836(A)

The Daresbury Laboratory has a vacancy at Higher Scientific Officer level for research into the

THEORY OF ATOMIC AND MOLECULAR PROCESSES

The fields of interest of the Theory Group at Daresbury are atomic and molecular, solid state and nuclear.

At the Laboratory a major nuclear structure facility is under construction and work will soon begin on a purpose-built X-ray and ultraviolet source. An IBM 370/165 computer is installed at the Laboratory, and a computational science group has recently been established working mainly in the quantum chemistry area.

The appointment would be a permanent one at Higher Scientific Officer level made according to age, qualifications and experience in the range £3,245 to £4,454. There are also pay supplements of (a) £313.20 per annum and (b) 5% of total salary subject to a minimum of £130.50 per annum and a maximum of £208.80 per annum. There is a non-contributory superannuation scheme and a generous leave allowance.

Applicants, male or female, must possess a good degree in an appropriate subject plus two years relevant experience, and preferably a Ph.D. in the area of atomic and molecular theory or expect to obtain one in 1978.

Closing date: 21 February 1978.

Please write enclosing curriculum vitae, the addresses of two referees and quoting reference number DL/608/T to:

The Personnel Officer

DARESBURY LABORATORY
Science Research Council
Daresbury, Warrington WA4 4AD

975(A)

BIOCHEMISTRY FACULTY POSITION

The Biochemistry Department, University of California, Riverside, invites applications for an Assistant Professor position (tenure track) effective Summer, 1978. Although we have a slight preference for a person with a strong background in physical biochemistry who will initiate research into the structure and function of biologically interesting macromolecules and/or biological membranes, applications from outstanding individuals in other areas of biochemistry will receive serious consideration. The successful candidate will participate in the department's instructional program at the undergraduate and graduate level. Candidates should include in their application a statement of their current and future research objectives and a statement of their teaching experience and areas of competence. Send curriculum vitae, complete bibliography, duplicate copies of three representative publications, and the names and addresses of three references to: BIOCHEMISTRY SEARCH COMMITTEE, Department of Biochemistry, University of California, Riverside, CA 92521. Applications will be accepted until March 10, 1978 or until a suitable candidate is located. The University of California is an Equal Opportunity/Affirmative Action Employer. Minority and women candidates are encouraged to apply. 968(A)

POSTDOCTORAL POSITION (Research Associate)

available for 1 to 2 years beginning January 15, 1978 or February 1, 1978, to work on luminescences of DNA and related compounds. Involves working with pulsed frequency-doubled laser and digital data acquisition system. Experience with lasers and digital electronics advisable. Send curriculum vitae and names of three references to: Dr Malcolm Daniels, Chemistry Department, Oregon State University, Corvallis, OR 97331. Oregon State University is Equal Opportunity/Affirmative Action Employer and complies with Section 504 of the Rehabilitation Act of 1973. Closing date for applications is January 15, 1978. 967(A)

DEPARTMENT OF NUTRITION AND FOOD SCIENCE MASSACHUSETTS INSTITUTE OF TECHNOLOGY FACULTY POSITION

The Department, a large multidisciplinary teaching and research unit with activities in all principal areas of nutrition and food science is seeking an applicant to fill the following faculty position:

An M.D., with research training and preferably with a Ph.D. degree, and experience and a major interest in human and clinical nutrition. The appointee will be expected to teach graduate level courses concerned with human and clinical nutrition, to develop a vigorous research programme and become actively involved in the research programmes of the M.I.T. Clinical Research Center.

The appointment will be at the Assistant or Associate Professor rank depending upon the qualification of the successful applicant.

Curriculum vitae, together with representative publications and the names of five referees should be submitted before February 1, 1978 to: Dr V. R. Young, Chairman Search Committee, Room 56-331, Massachusetts Institute of Technology, Cambridge M.A. 02139, U.S.A.

M.I.T. is an Equal Opportunity Employer, Representatives of minority groups are urged to apply. 830(A)

CURATOR

THE GILBERT WHITE AND OATES MUSEUMS

seek full- or part-time Curator. Details: The Wakes, Selborne, Hampshire. 1029(A)

FACULTY POSITION IN BIOCHEMISTRY UNIVERSITY OF ILLINOIS AT URBANA CHAMPAIGN

The Department of Biochemistry is seeking applicants for a tenure track appointment at the level of assistant professor or higher to begin August 1978. Applicants should be qualified to teach biochemistry courses at undergraduate or graduate levels. Only candidates who have demonstrated excellence in research and who are prepared to establish an active research program will be considered, preference will be given to those who have received graduate and post-doctoral training in strong research environments. The minimum salary at the assistant professor level is \$15,000 for a nine-month appointment; salaries at higher ranks are negotiable. For full consideration, application material, including a curriculum vitae, and list of publications, a brief description of research interests and three letters of reference should be sent by February 1, 1978.

Professor Lowell P. Hager, Head
Dept. of Biochemistry
University of Illinois
Urbana, Illinois 61801
Telephone: (217) 333 3945
The University of Illinois is an affirmative action/equal opportunity employer. 785(A)

FELLOWSHIPS

IMPERIAL CANCER RESEARCH FUND DEPARTMENT OF CELLULAR CHEMOTHERAPY Research Fellowship

A postdoctoral cell biologist, biochemist or pharmacologist with previous experience or interest in *in vitro/in vivo* cloning techniques and/or cancer chemotherapy is required to join a group working on effects of antitumour drugs in normal and malignant cell systems, assessing the value of timing and sequencing of drugs in combination schedules, with particular emphasis on the design of safe combinations in terms of their effects on human bone marrow function.

Appointment will be for two years in the first instance with possible extension for a third year. Salary with entry according to qualifications and experience within range £4,649 to £5,669.

Further information from Dr Bridget T. Hill (tel: 01-242 0200 extn. 397). Applications with curriculum vitae and names of two referees, should be sent to The Secretary, Imperial Cancer Research Fund, Lincoln's Inn Fields, London WC2A 3PX by January 27, 1978. 978(E)

POSTDOCTORAL FELLOWSHIPS DEPARTMENT OF PHYSICS UNIVERSITY OF WATERLOO

Waterloo, Ontario, Canada

Applications are invited for post-doctoral research in physics

Fellowships carry a stipend of \$11,220 per annum. The awards are tenable for the period of one year with a possible renewal for a second year.

Research areas include:

Atomic and Molecular Physics, Biophysics, Brillouin Spectroscopy, Crystallography, Electron Microscopy, Electron tunneling, Ellipsometry, Experimental superconductivity, Diffraction Physics, Far-infrared spectroscopy, Infra-red and Raman Spectroscopy, De Haas-van Alphen effect, Laser Physics, Lattice dynamics, Magnetoconductivity of metals, Microwave Collision-Induced Absorption and Interferometry, Nuclear magnetic resonance, Semiconductors and amorphous semiconductors, Transport properties of gases and intermolecular potentials, Theoretical studies; magnetic impurities in superconductors, relativistic effects in metals, electronic structure in metals, nonlinear behaviour of elastic dielectrics, lattice dynamics of ionic crystals.

The applicants should submit their résumés and the names of three referees to:

Dr C. C. Lim
Department of Physics
University of Waterloo
Waterloo, Ontario, Canada
N2L 3G1 929(E)

SYMPOSIUM

MICROCOSMS IN ECOLOGICAL RESEARCH

November 8-11, 1978—Augusta, Georgia, U.S.A.

Savannah River Ecology Laboratory, in cooperation with University of Georgia's Institute of Ecology; U.S. Dept. of Energy; National Environmental Research Park program; U.S. E.P.A.'s Environmental Research Lab, Athens, Ga.; and, Electric Power Research Institute's Environmental Assessment Dept., will host ecological symposium on use of microcosms in ecological research. Symposium format will include presentations by invited speakers, panel discussions, and contributed papers. Abstracts due July 1; manuscripts due September 1, 1978. For registration and additional information contact: Dr John P. Giesy, Jr, Symposium, Savannah River Ecology Laboratory, Drawer E, Aiken, S.C. 29801, U.S.A. 1000(M)

AWARDS

THE WELLCOME TRUST 1 Park Square West, London NW1 4LJ Telephone: 01-486 4902 AWARDS FOR TROPICAL GASTROENTEROLOGICAL RESEARCH

The Wellcome Trust wishes to encourage research into major diseases of the gastrointestinal tract occurring in the tropics. Applications from gastroenterological or other appropriate departments will be considered. Preference will be given to proposals which demonstrate an interdisciplinary approach and especially those which include sustained collaboration with research centres in the tropics.

Competitive awards of up to £100,000 each are offered for a period of not more than five years. These awards are additional to the Trustees' normal arrangements for supporting tropical research.

Those wishing to apply should send a two-page summary of their objectives and proposals for research including a brief estimate of the anticipated costs for preliminary consideration. The summary should be accompanied by the curriculum vitae of the principal scientist involved. Applications should be addressed to Dr B. E. C. Hopwood, The Wellcome Trust, 1 Park Square West, London NW1 4LJ, and reach the Trust by March 21, 1978. 999(N)

RESEARCH FELLOWSHIP

Postdoctoral Fellowship in Human Cell Biology tenable for two years in the first instance with possible extension for a third year, available from January 1978 for studies on the regulation of DNA, RNA and protein synthesis and on the expression of breast specific function in primary cultures of human normal and dysplastic breast tissue. Candidates should be experienced in cell culture and in the biochemistry of growth regulation.

Salary with entry according to qualifications and experience within range £4,649 to £5,669.

Further information from Dr R. Hallows (tel. 01-242 0200 ext. 307). Applications with curriculum vitae and names of two referees, should be sent to The Secretary, Imperial Cancer Research Fund, Lincoln's Inn Fields, London WC2A 3PX by January 31, 1978 (Quoting ref. 33/78). 984(E)

DEPARTMENT OF MICROBIOLOGY DALHOUSIE UNIVERSITY HALIFAX, NOVA SCOTIA, CANADA

Applications are invited for POSTDOCTORAL FELLOWSHIPS to study viral pathogenesis with special emphasis on the role of interferon. Fellowships beginning July 1, 1978, are available for a period of two years with an initial stipend of \$12,720 per annum. Candidates should have research experience in the field of animal virology, preferably with an interest in the biochemistry of nucleic acid biosynthesis. Applications, consisting of curriculum vitae and names of two referees, should be sent no later than March 30, 1978 to Dr K. R. Rozee, Professor and Head, Department of Microbiology, Faculty of Medicine, Dalhousie University, Halifax, Nova Scotia, Canada. 932(E)

MRC

Medical Research Council

Training Fellowships Scheme 1978-79

Tenable in the U.K.

The Council invites applications from suitably qualified candidates for MRC Training Fellowships.

Eligibility Requirements—

(candidates should have been ordinarily resident in the UK throughout the period of 3 years immediately preceding the application date)

(1) Graduates in medicine

(2) Graduates in dentistry

From post-registration up to Senior Registrar or equivalent at academic levels.

(3) Graduates in science

With PhD or D Phil, or with MSc plus at least 3 additional years' postgraduate experience; up to maximum seniority for a non-clinical academic lecturer.

Training Available

For (1), (2) and (3): specialised research training or training in the basic subject relevant to a particular clinical interest.

For (1) and (2) at House-Officer to Registrar or equivalent clinical academic level: a suitable MSc or comparable course.

Stipends

For (1), (2) and (3): appropriate point, with increments, on the current NHS medical (House Officer to Senior Registrar) equivalent dental, or current relevant academic scales.

Tenure

From 6 months to 3 years (or duration of MSc course).

Further information and application forms are available from: Medical Research Council, Training Awards Group, 20 Park Crescent, London W1N 4AL (tel. 01-636 5422 ex. 240).

CLOSING DATE FOR APPLICATIONS: 28 FEBRUARY 1978

Late applications cannot be considered.

986(E)

University of St Andrews Research Fellow in Statistical Computing

Applications are invited for a research fellowship to review and develop further statistical methods for exploratory data analysis. Postgraduate research experience is required, with a strong background in statistics. A working knowledge of computer methods and statistical software is essential.

The appointment will be for 2 years in the first instance, with the possibility of extension. Salary on Research Range 1A £3,333 to £5,627 plus U.S.S., starting point depending on qualifications and experience.

Applications (2 copies) including a curriculum vitae and the names of 3 referees should be sent by February 24 to Mr M. Weatherill, Department of Computational Science, University of St Andrews, Fife KY16 9SX, from whom further particulars may be obtained.

992(E)

UNIVERSITY OF MELBOURNE POSTDOCTORAL RESEARCH FELLOWSHIP in the DEPARTMENT OF MICROBIOLOGY

Applications are invited from Ph.D. graduates for the above-mentioned position which is funded by the Australian National Health and Medical Research Council and is available from January 1978 or soon after. The candidate will participate in a programme to develop continuous cell lines derived from immune lymphocytes. Previous experience in cytology, especially in the use of fusion hybrid techniques, would be an advantage. The position is available for two years with the possibility of renewal.

SALARY: \$A17,279 per annum. Enquiries will be welcomed by Dr W. Boyle, Department of Microbiology, University of Melbourne, Parkville, Victoria 3052, Australia. Applications referring to position number 526 355 should be addressed to The Registrar, University of Melbourne, Parkville, Victoria 3052.

1011(E)

UNIVERSITY OF BIRMINGHAM DEPARTMENT OF CHEMISTRY POSTDOCTORAL RESEARCH FELLOWSHIP POSTGRADUATE RESEARCH ASSOCIATESHIP Molecular Dynamics of Photodissociation

Applications for two posts supported by S.R.C. are invited to undertake research in collaboration with Dr J. P. Simons. The experimental techniques to be employed include high resolution photo-fluorescence spectroscopy in the near and vacuum ultraviolet and time-of-flight photofragment spectroscopy using rare gas-fluoride ultra-violet laser sources.

The postgraduate position is tenable for three years and the holder will be encouraged to pursue research leading to a higher degree: the postdoctoral position is tenable for a maximum of two years: both can commence on or before October 1, 1978.

Salaries: Fellowship on the scale £3,333 to £5,627, (under review), plus superannuation. Maximum starting salary £3,761.

Associatehip on the scale £2,904 to £4,190 (under review), plus superannuation. Maximum starting salary £2,904.

Further particulars from the Assistant Registrar, (Science and Engineering), University of Birmingham, P.O. Box 363, Birmingham B15 2TT, to whom applications (three copies) with full c.v. and naming three referees should be sent by Friday, February 24, 1978. Please quote ref: NF4.

971(E)

COURSES



FLOW LABORATORIES PLANT TISSUE CULTURE COURSE

to be held in Edinburgh, April 3 to 7, 1978. The Course will be run by Dr M. M. Yeoman and will consist of lectures, experiments and demonstrations of the techniques involved in the preparation of explants, the production and sub-culture of callus cultures, organ culture, protoplast production and culture, and the induction of organogenesis.

The fee for the Course will be £135 plus VAT inclusive of accommodation.

Application forms are available from Dr S. A. S. Volkers, Flow Laboratories Ltd., P.O.B. 17, Second Avenue, Industrial Estate, Irvine, KA12 8NB, Ayrshire.

1023(D)

UNIVERSITY OF SALFORD DEPARTMENT OF BIOLOGY Applications are invited for a Science Research Council RESEARCH ASSISTANTSHIP

to work with Dr V. W. Pentreath on nerve/glia relationships in invertebrate nervous systems. The appointment, of three years duration, will be made up to Postdoctoral (Grade 1A) level. Applicants should preferably have experience with electron microscopy and/or autoradiography.

Applications with curriculum vitae and two academic references should be sent to the Registrar, University of Salford, Salford M5 4WT, by January 31, 1978, quoting Reference B/72/N.

1022(P)

IMPERIAL COLLEGE OF SCIENCE & TECHNOLOGY ROYAL DUTCH SHELL SCHOLARSHIP IN GEOLOGY OR GEOPHYSICS 1978-1979

Normally of equivalent value to a N.E.R.C. Postgraduate Studentship plus supplementation, plus college fee and approved fieldwork expenses. Tenable for up to three years of research in a topic which falls within Shell's general field of interest. Candidates must have taken, or be taking in the year of award, either an honours degree in Geology, preferably with mathematics and/or Physics at an ancillary level, or an honours degree in Physics. Candidates should be no more than 27 years of age, and medically fit to work abroad.

Applications, on a special form obtainable from the Registrar, Imperial College, London SW7 2AZ must be received by April 14, 1978.

694(H)

The Macaulay Institute for Soil Research, Aberdeen

RESEARCH STUDENTSHIPS

Applications are invited from students who hold or expect to hold upper second class honours degrees in the appropriate subject for Ph.D. studentships at the standard S.R.C./A.R.C. rates for work in the following areas:

Spectroscopy (E.S.R. and Laser)
Physical Chemistry (Sorption and Thermochemistry)
Organic Chemistry (Enzymes)
Plant Physiology (Calcium translocation)

Candidates, who must have British nationality, should submit applications to Professor T. S. West (Director), The Macaulay Institute for Soil Research, Craigiebuckler, Aberdeen AB9 2QJ.

987(F)

CONFERENCES

ELECTROPHORESIS '78

An international conference and poster presentations will be held at the MIT Krege Auditorium in Cambridge, Massachusetts, April 19-21, 1978. More than twenty invited speakers will present lectures on all types of electrophoretic and isoelectric focusing methods and applications to proteins, nucleic acid and cell separation. Abstracts for poster presentations must be received by March 1, 1978. Pre-registration is required. For brochure and registration forms write to:

Prof. Nicholas Catsimopoulos
MIT, Room 56-307
Cambridge, Massachusetts 02139

952(C)

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For high performance gel filtration

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see you tomorrow -
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n packing times. Sephacryl is pre-swollen ready-to-use in packs of 750 ml and

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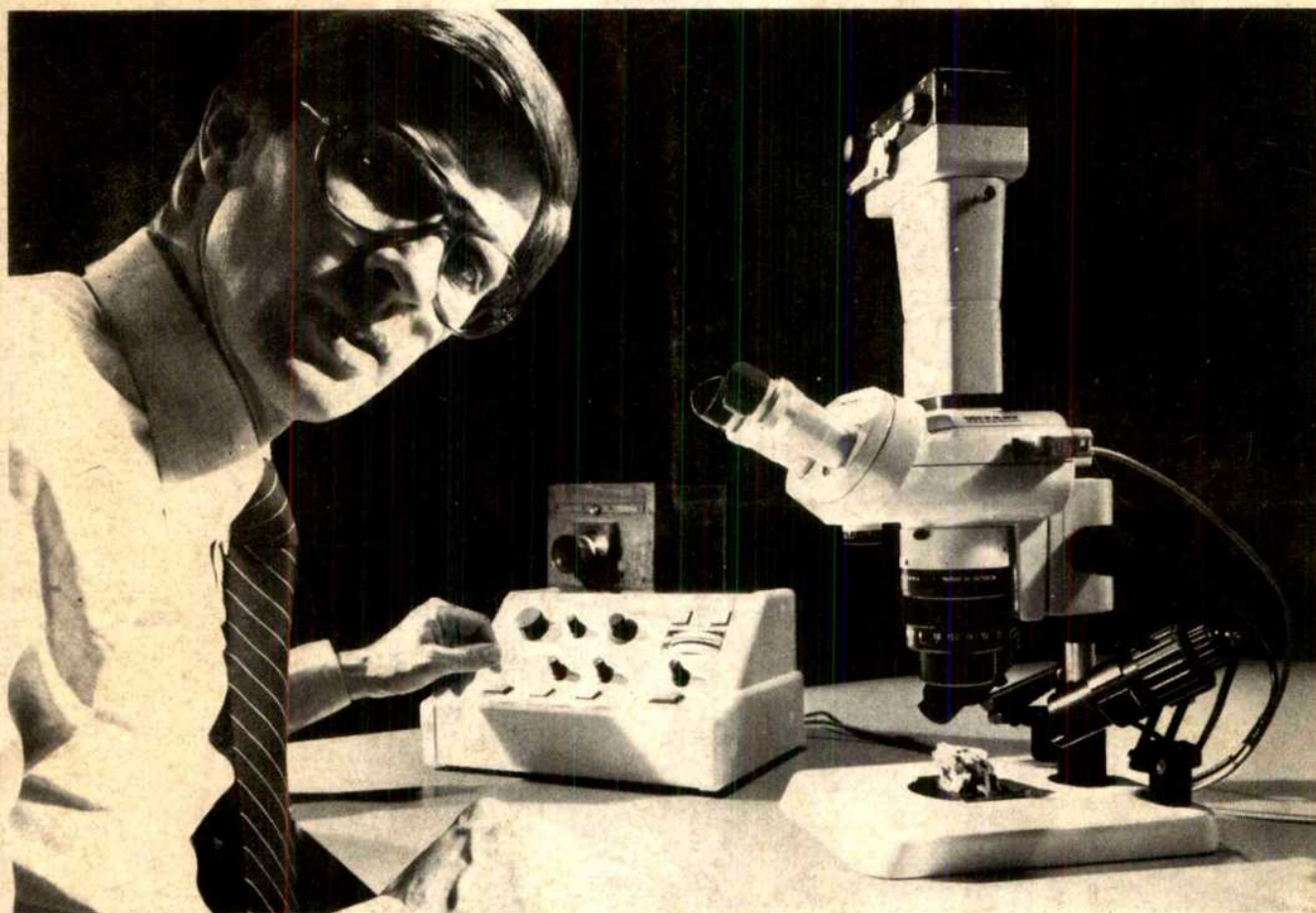
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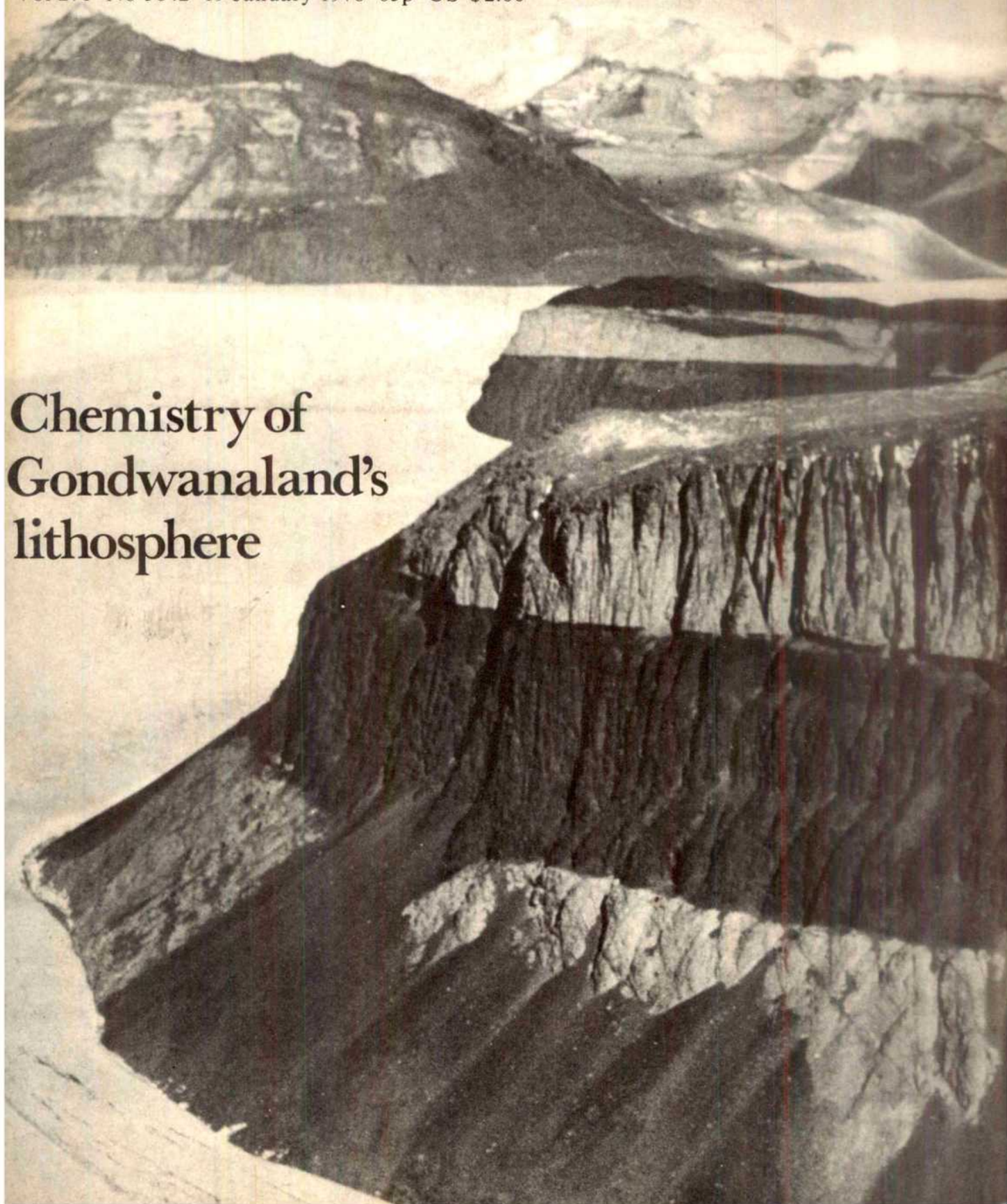
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Published weekly
ISSN 0028-0836

Registered as a newspaper at the
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1976 Index (post-paid) U.K. £2.50. Rest of
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Cover picture

Ferrar dolerite (dark), intruded into
Beacon Sandstone in the Asgard
Range of East Antarctica, is used by
Drs Brookes and Hart (page 220) in
a study of the chemistry of the early
lithosphere.

[Courtesy of L. Jones]

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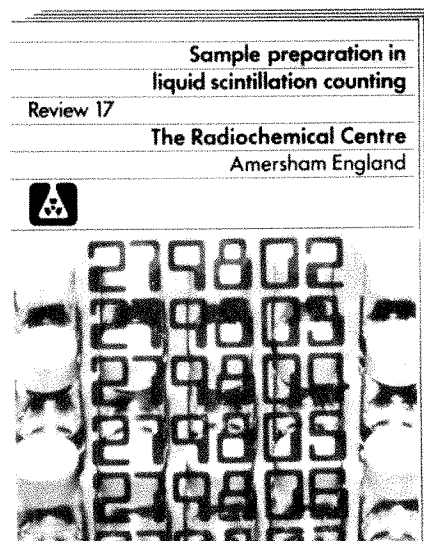
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nature*19 January 1978*

Cinderella in the UN

MANY of those in touch with the preparations for the UN Conference on Science and Technology for Development (UNCSTD), now scheduled for Vienna in August 1979, have been concerned with the apparently low priority given to possible contributions from the world scientific community. Recently, however, there would seem to have been a change of heart, if not within the UN's own Office of Science and Technology, at least on the part of Mr da Costa, the Conference's Secretary General.

This much became apparent at the meeting in Geneva last November of the Advisory Committee on the Applications of Science and Technology, ACAST, the United Nations' informal committee. ACAST was one of the few concrete results of the last big general conference on the Application of Science and Technology for the Benefit of Less-developed Areas (UNCSAT) held in 1963. Rather to the surprise of ACAST's members, who had previously found him somewhat inclined to play down the part that scientists might play in 1979, da Costa went to some pains to stress the important contribution they could and should make not only at the Conference itself, but also in the preparation of the national papers that will provide the principal input to UNCSTD. He spoke, too, of the "necessity to ensure the participation of scientists at the forthcoming session of the UNCSTD Preparatory Committee", which "will allow them to speak alongside diplomats at a very early stage in the Conference preparations".

In fact, competition for the floor of meetings is less likely to be between scientists and diplomats than between scientists and 'experts' in the development business who represent, in UN terms, the UNCTAD and UNIDO lobbies.

The UN is still agonising about whether it should have a science and technology policy at all. This is the subject of an important report from ACAST that will be considered by the Committee on Science and Technology for Development (CSTD—the secretariat of UNCSTD) at its meeting which starts on 23 January in Geneva. While ACAST itself believes that "there is a need for a policy which would guide and facilitate the development and application of science and technology by and within the United Nations system for the benefit of its Member States" the various members of the UN family, and in particular the specialised agencies, seem

decidedly lukewarm if not frankly antagonistic to this idea. The fact is that, although themselves almost without exception based to a greater or less extent on science or its immediate applications, they are notoriously jealous of their own sovereignty in matters of policy—indeed, it sometimes seems that this is almost the only thing on which they can agree.

Nonetheless, if the agencies which purport to advise and assist the developing countries in applying science and technology cannot agree at least to certain priorities in this field, much of their effort will be wasted and the developing countries themselves will still lack the guidance they so desperately need on where to go and how to set about getting there. In this context, the new report from ACAST is well worth careful study and consideration, as much outside the UN system as within.

As to ACAST itself, after a period when, again within the UN system, its very usefulness has been called in question, the recent meeting should have dispelled any doubts as to its value. Its members do not sit as official government representatives but in their own right as scientists of international repute, and covering a very wide range of disciplines. This also seems to have been recognised by Mr da Costa when he stressed the value of ACAST's role in "acting as a bridge between the Conference Secretariat and the scientific community" particularly with regard to the participation in 1979 of the major non-governmental organisations such as ICSU, the Pugwash Conference and the World Federation of Engineering Organisations (WFEO), all of whom found it worthwhile to send observers to the ACAST meeting.

One point which may be of concern to British scientists is that at present no scientists from this country is a member of ACAST. This is partly because in inviting scientists to sit on this Committee, the UN Secretary General seeks to avoid the impression of any permanent 'national' representation. In part, too, even ACAST is affected by the move to include more representation of the developing countries among its members (in fact the Chairman of the recent meeting was Dr Chagula of Tanzania). Be that as it may, there is little doubt that when, as will surely happen sooner or later, there is any suggestion that a British scientist should once again be invited to sit on this, the only UN body that can speak for science and scientists, it should be warmly welcomed. □

Is British physical anthropology dying?

Bernard Campbell, Adjunct Professor of Anthropology, University of California, Los Angeles, argues that teaching and research in physical anthropology should be developed and expanded in the UK.

RESEARCH and teaching in the most fundamental branch of the science of physical anthropology is today almost extinct in Great Britain. Senior positions are no longer available, and almost all the talented scholars in the field have moved to the US or Canada. Human palaeontology, as a key part of the anthropological sciences, is taught by permanent staff in the Anthropology Department of only one University: Cambridge. While it is also taught in a limited way in at least five anatomy departments of medical schools, this teaching is generally in the context of human anatomical studies and is therefore not in its most appropriate milieu, namely, the knowledge of human society and culture, and of the evolutionary paradigm.

This is a near disaster. Physical anthropology, concerned as it is with man's biology, is the study of the most fundamental aspects of human nature. While it can be said to date from J. F. Blumenbach (1752-1840) (who classified races and made a collection of human skulls) it came of age and gained its central paradigm with the publication of Darwin's *Origin of Species* (1859).

Today, physical or biological anthropologists study Man as a product of natural selection and try to understand human variability in the light of evolution. The biology of Man has branched into many sub-disciplines, including comparative anatomy and comparative physiology, which are at the centre of human biology; the study of human growth and variability; genetics; primate palaeontology; primate behaviour; human ecology; and many others. Physical anthropology is therefore necessarily disparate in its specialisations.

At University College, London, for example, staff are presently doing research into the systematics of the *Galagidae*, the genetics of marriage in modern Britain, the serological genetics and nutrition of Carib populations, the genetics of Ethiopian baboons, and the strength and structure of bone. Many of these specialisations lean heavily on developments in related sciences, especially human genetics and physiology, and zoology. Sometimes it seems that these diverse specialists would be better placed in other departments. Yet research into human blood-groups was immensely stimulated by anthropological considerations and data, and studies of primate behaviour were given a new focus by anthropologists S. L. Washburn and I. DeVore. Anthropologists have both received from, and given to, related disciplines, and their central interest in 'the proper study of mankind' energises and directs their research.

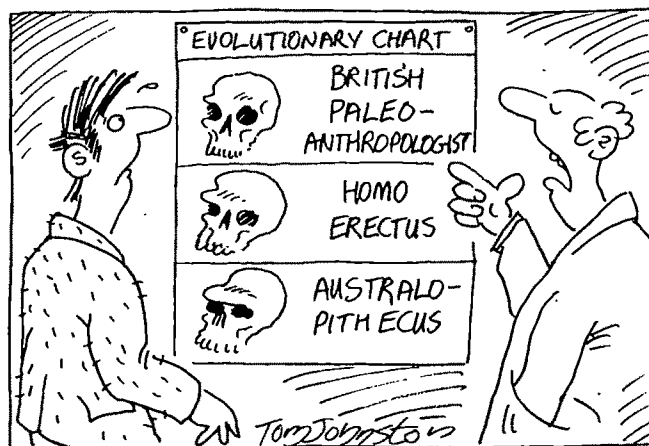
In the early years of this century Karl Pearson, G. M. Morant and others, worked painstakingly with calipers and measuring tapes to produce a vast amount of quantitative data about the skeleton of modern man. They also studied the rather limited fossil record in the same way. The method produced meagre results and in the 1940s Fisher's population genetics and the new research on the ABO blood groups developed as a far more powerful tool with which the nature of racial differences and at least recent human prehistory might be uncovered. The dimension of time proved elusive, however, and while the genetical aspects of Man's place in nature probably hold pride of place today in most departments of physical anthropology throughout the world, these studies do not and cannot develop that central explanatory paradigm, which alone can hold physical anthropology together as a science.

The fossil evidence for human evolution and its interpre-

tation into a more complete understanding of Man's past has become alive again with the development of a functional approach to anatomical analysis. S. L. Washburn was probably the first to insist on its importance (circa 1950) and others followed. My book *Human Evolution* (1966) was an early attempt to make such a functional integration. The bones came to life, and behavioural speculation followed. As a result physical anthropology came closer to its sister discipline, prehistoric archaeology: together they constitute the science of evolutionary anthropology or palaeoanthropology *sensu lato*. Palaeoanthropologists are not today expected to be outstanding scholars in both human palaeontology and prehistoric archaeology, but they are expected to be very much aware of the value of each science and its vital importance in understanding the evolution of human nature. The interdisciplinary study of the evolution of man which also includes vertebrate palaeontology, palaeoecology, and palaeogeography, is necessarily the main stem from which all the sub-disciplines of physical anthropology mentioned above are nourished. In spite of the complexity of anthropology, an anthropologist must necessarily be a generalist as well as a specialist: he must specialise in the light of his knowledge of mankind's whole adaptive strategy.

Human palaeontologists must, therefore, more than ever before, rub shoulders with social and cultural anthropologists and archaeologists: and at the same time they must see man at all times—not as a creature apart from nature, but as a very remarkable animal. It follows that although the anatomy departments have housed and nourished human palaeontologists for three to four generations in the UK (since the time of Sir Grafton Elliot Smith and Sir Arthur Keith), they can no longer remain the primary resource for this subject.

At a recent inaugural meeting in London, the L. S. B. Leakey Foundation presented its goal of further study into "Man's origin, behaviour and survival", and plans to support students and research projects in areas which reflect this holistic approach to the study of man. What is needed in Britain is a Department of Physical Anthropology with the prestige, money and manpower to bring back to Britain some of those palaeoanthropologists who have left. Britain needs a university with the will and the resources to bring to life the main stem of all anthropology—the study of human evolution. Man has been part of Nature—as a hunter and gatherer—for 99% of the approximately 2 million years of his existence on earth. Man is a product of his prehistory, and our understanding of our present predicament depends squarely on our knowledge of our own past. □



"Of course, they're extinct now too!"

Research with prisoners

After 18 months' deliberation the US Secretary of Health has responded to a report questioning the ethics of research involving prisoners. **Michael S. Yesley** and **Barbara Mishkin**, closely involved in the production of the report, here discuss their findings

UNDER American law new drugs must undergo three phases of testing before being approved by the Food and Drug Administration (FDA) for general sale. Phase 1 involves the first introduction of a drug in humans and requires testing on normal volunteers to determine the safety and general metabolic properties of the drug. Phase 2 consists of controlled clinical studies on a small number of patients to determine therapeutic efficacy. (In the United Kingdom and elsewhere, phases 1 and 2 are consolidated by testing for safety as well as efficacy on a small number of patients for whose treatment the drug is intended.) Phase 3 is similar to phase 2 but involves considerably more patients. Although no data are available by which to ascertain the extent of the use of prisoners in phase 1 tests in the United States, it is clear that they are used to a significant degree.

The reasons for the practice are partly historical. During the Second World War, civil prisoners in Illinois and New Jersey participated in research to develop treatment for malaria and other diseases that afflicted the armed forces. Such participation was considered not only acceptable but praiseworthy, for it gave inmates an opportunity to contribute to society (a form of restitution) and to enhance the war effort. Following the war, the involvement of prisoners in non-therapeutic biomedical research increased as a result of expanding government support for biomedical research and more stringent federal requirements for evaluating the safety of new drugs.

At the same time that the use of prisoners was increasing in the United States, the world was reacting to revelations of the experiments conducted in the Nazi prison camps. The Public Health Council of the Netherlands, for example, responded by specifically disapproving research involving children, old people, the insane and prisoners. The Nuremberg Code, initially drafted by an American consultant to the war crimes tribunal, is somewhat ambiguous with regard to the involvement of prisoners in research. The first principle of the Code provides that: "The voluntary consent of the human subject is essential. This means that the person involved should have legal capacity to give consent: should be so situated as



Self-confidence training at Wisconsin State Reformatory

to be able to exercise free power of choice without the intervention of any element of force, fraud, deceit, duress, overreaching, or other ulterior form of constraint or coercion. . . ."

Many have interpreted this provision to mean that civil prisoners may not participate as research subjects, because they are not "so situated as to be able to exercise free power of choice." In the United States, however, it has been argued by the research community and by some prisoners themselves that so long as the prisoners are fully informed and no obvious duress is imposed, they may be considered free volunteers. This assertion is at the centre of the recent controversy in the United States over the participation of prisoners in research.

Jessica Mitford's book *Kind and*

Usual Punishment, published in 1973, was partly responsible for calling the public's attention to this issue. Mitford perceived drug research to be an exploitation precipitated by FDA drug-testing requirements and perpetuated by the economic self-interest of drug firms, investigators, prison authorities and the inmates themselves. Concurrently, academicians and civil rights organisations began to question the validity of any consent given by prisoners to participate in nontherapeutic research. They suggested that voluntary consent was impossible in penal institutions because of the poor living conditions, fear of physical violence in the cell-block, absence of good medical care and alternative ways to earn money in prison, and the obvious advantages, therefore, of leaving the general prison quarters to be paid for living in a research unit with access to individuals from the outside world. They also suggested that in a closed society such as a prison, the opportunity for exploitation is especially great when, as Mitford pointed out, prisoners are frequently prevented from reporting abuses to those on the outside.

Proponents of prison research have argued, on the other hand, that if research in prisons were to be prohibited, the result would be an inability to assure the safety of new pharmaceuticals, since prisoners are most likely to have the time and inclination to subject themselves to the kind of tests required by FDA to establish safety of new drugs. The proponents state, in addition, that phase 1 drug testing presents little risk, that injuries have been few, and that procedures are available to assure that consent is freely and knowledgeably given without undue influence or coercion.

In the midst of this debate, the National Commission for the Protection of Human Subjects of Biomedical and Behavioural Research was established by Congress to conduct studies and make recommendations in several areas of controversy involving

Drs Yesley and Mishkin are Staff Director and Assistant Staff Director, National Commission for the Protection of Human Subjects of Biomedical and Behavioral Research, Washington, D.C., but the views expressed herein are those of the authors and do not necessarily reflect those of the Commission.

human subjects, including the participation of prisoners. The Commission set out in 1975 to investigate the validity of the various claims put forth and to examine the legal and ethical issues surrounding the participation of prisoners in research. The members of the Commission and its staff visited several prisons where research is conducted and interviewed over 250 prisoners who participate in research. The Commission conducted public hearings at which arguments for and against prison research were presented, and sponsored a conference at which the view points of minority populations were expressed. In addition, several papers and studies were prepared for the Commission on topics including the nature and extent of prisoner involvement in research in U.S. prisons; alternatives to the use of prisoners and foreign practices in drug testing; philosophical, legal and sociological perspectives on the involvement of prisoners in research; experimental behavioural practices involving prisoners; and a sociological study of research at five prisons.

The Commission found that virtually all prisoners participating in biomedical research feel that they do so freely and do not want to lose this opportunity. Most prisoners who were interviewed candidly cited financial reward as the main reason for their participation in research, and they did not appear to be motivated by the expectations of early release (which, in fact, is not granted for research participation). Further, data indicate that even in prisons where the inmate population is predominantly black, the research subjects are predominantly white, and they tend to be better educated and to hold better prison jobs than the nonparticipants. This finding contradicts the general belief that prison research exploits minority groups and those unable to obtain good prison jobs (to the extent such employment is available).

The Commission was impressed with reports of programmes that successfully use nonprisoner volunteers in phase I drug testing. At least one principal investigator (who formerly used prisoners for such research) reported that nonprisoner volunteers are preferable for scientific as well as social and ethical reasons. It is easier to control the subjects' abuse of drugs, for example, in a well-run research facility than in a prison. Further, he reported, the subjects become experienced in the bargaining and consent process, they are knowledgeable about the responsibilities and risks they are undertaking, and it is possible to provide commercial insurance to compensate them for any injuries that might occur.

With respect to the risks of phase I



Learning to concentrate by crawling

drug testing, it appears that they are not significant. One commercial company that provides insurance for non-prison volunteers has estimated that the risk of such "employment" is roughly equivalent to that of being a secretary.

This situation, in which the prisoners expressed their desire to participate in research, while advocates and other outside observers argued that the prisoners were unduly influenced, posed a dilemma for the members of the Commission. The irony was not lost that recognition of the limited options in prison life might be used to justify the removal of one of those options. On the other hand, it is likely that many prisoners who participate in research would choose not to do so if the conditions of prison life were substantially improved or there were optional sources of equivalent income. This likelihood may indicate undue influence, and thus involvement of prisoners in research may well constitute an exploitation.

Despite the confidence of prisoners that their participation in research was entirely voluntary, the Commissioners expressed doubts that conditions generally prevalent in United States prisons provide a setting in which voluntariness can be relied upon. The Commissioners also expressed reservations about the

conduct of research in institutions that are isolated from public scrutiny and in which access to the outside is limited. The Commission concluded that the conduct of nontherapeutic research should be permitted only under acceptable prison conditions and that the burden of proof as to the social necessity of using prisoners as subjects in nontherapeutic research should be on those who wish to continue the practice.

The Commission therefore recommended to the Secretary of Health, Education, and Welfare and to Congress that the participation of prisoners in nontherapeutic research not be permitted unless any prison proposed as a site for such research is able to meet a long list of specific standards regarding health and safety conditions, job opportunities, free communication with persons on the outside, prisoner representation on research review boards, and the right of prisoners to form effective grievance committees. In addition, any investigator proposing to use prisoners in such a facility must demonstrate that the need to use prisoners in a particular research project is compelling and that such use would not result in social inequity.

The Commission recommended, however, that research designed to improve understanding about the causes of deviant behaviour, the effects of incarceration, and the factors related to parole performance and recidivism be permitted, so long as certain procedures are followed to protect the rights of prisoners, to safeguard their privacy and to preserve the confidentiality of the data obtained.

The Commission's recommendations* were submitted to the Secretary of Health, Education and Welfare in October 1976. After a delay occasioned by disagreement in the department over the wisdom of implementing the recommendations, Secretary Joseph Califano, Jr., announced last month that he would issue regulations even stricter than those recommended by the Commission for nontherapeutic research with more than minimal risk (e.g., phase I drug testing). In fact, he proposed to ban the participation of prisoners in such research under any conditions. The Secretary's stated grounds for his decision espoused the arguments of civil rights advocates that prisoners, by the nature of their situation, are incapable of giving informed consent. □

*Copies of the Commission's report and recommendations may be obtained by writing to: Public Information Officer, National Commission for the Protection of Human Subjects, Room 125, 5333 Westbard Avenue, Bethesda, Maryland 20016.

Fermilab's director threatens resignation



FOR a considerable number of years, Europe's high energy physicists have looked enviously at the facilities and resources available to their US colleagues. Recently, however, the pendulum has begun to swing the other way; American physicists, faced with rising costs and conflicting demands on a limited budget, now speak of the need to "keep up with the Europeans".

The most dramatic manifestation of this has been the suggestion by Dr Robert R. Wilson, director of the Fermi National Accelerator Laboratory near Chicago, that he will resign his post next month unless the Government provides sufficient funds for early completion of the laboratory's proposed 'energy doubler', a development which would both cut down running costs and allow the laboratory to reach energies of 100 GeV or more.

Wilson stated his position in a letter to Mr James R. Schlesinger, Secretary for Energy, whose department is responsible for high energy physics laboratories. Schlesinger's proposed budget for 1979 will be presented to Congress next week by President Carter. And although regarded by some as an attempt at special pleading, Wilson's move has highlighted tensions over the allocation of funds within the high energy physics community.

Fermilab—the "jewel in our crown" as one member of this community puts it—was completed on time and within budget (\$340 million) in 1972. Although designed to carry out experiments at 200 GeV, it has been operating at energies of up to 500 GeV, and was for several years the most powerful particle accelerator in the world. Much important and exciting physics has come out of the laboratory. Only last year, for example, Fermilab announced the dis-

covery of the new ψ particle of mass about 10 GeV. This particle is believed to be similar to the J/ψ at 3.1 GeV and to herald the presence of a new quark.

Yet it has been estimated that the new SPS proton synchrotron facility at the European Centre for Nuclear Research (CERN) in Geneva, which came into operation at the beginning of last year and currently operates at about the same energy at Fermilab, enjoys almost twice the level of support from research teams financed by the different European countries involved. As a recent article in the Fermilab house journal put it "whereas we welcome the arrival of our friends and co-investigators in the field of 400-GeV physics, we also must be mindful of the competition. There is no point in doing an experiment at Fermilab, quite similar to one being done at CERN, and doing it less well."

"At present, we are doing only about half the amount of physics a year that we could be doing" according to Dr Edwin L. Goldwasser, deputy director of the laboratory. "And in two or three years time, if there is no improvement in funding, we could find ourselves in a relatively weak position internationally." Already it is planned that Fermilab's meson facility is to close for six months for lack of funds.

Saving energy—and thus reducing Fermilab's \$5 million electricity bill—is therefore one of the attractions of the "doubler" project, under which a second ring of superconducting magnets would be constructed using the same tunnel as the existing ring.

According to Dr Goldwasser, a whole new set of experiments would be opened up by such a facility, which would be able to provide proton energies on a target of up to 1000 GeV (43 GeV in the centre of mass). And by circulating particles in opposite directions in the two rings—for example protons and antiprotons—collision energies of 2000 GeV in the centre of mass, at high luminosity, could be reached.

With the major part of the development work carried out, Wilson is now keen to move forward rapidly to the construction phase. "At present the doubler would cost between \$30 and \$35 million, and given this money—which would mean a total budget for the laboratory of about \$120 million in 1979—we believe that the doubler could be completed by the end of 1979" says Dr Goldwasser.

Members of the Department of Energy's High Energy Physics Advisory

Panel, who would recommend the funding, are sympathetic to Wilson's position. There is a widespread feeling that, despite new facilities and continuously increasing budgets, the overall position of high energy physics is precarious. According to Professor Sidney Drell, for example, who is chairman of HEPAP and deputy director of the Stanford Linear Accelerator Center (SLAC) in California, the present level of funding is "perilously low", with virtually all major equipment being underutilised.

In this climate, there is a natural concern that Fermilab should continue to operate effectively and competitively. And HEPAP has suggested that, for the time being at least, the administration should hedge its bets and continue to support all its major accelerator facilities. But the question has become one of deciding relative priorities.

In particular HEPAP has been faced with the choice between upgrading Fermilab's facilities by constructing the "doubler" as Wilson suggests, and providing support for a new facility, the 400 GeV intersecting proton beams project (Isabelle) at the Brookhaven National Laboratory on New York's Long Island.

At present, while emphasising that it is "strongly committed" to both projects, HEPAP's vote for top priority has gone to Isabelle, just as five years ago it recommended that funds for other areas of high energy physics be held back to allow Fermilab to be developed.

HEPAP's position was confirmed at a meeting of the panel attended by both Wilson and Goldwasser in early December (and planned, ironically, to coincide with the opening of a new gallery devoted to particle accelerators in the Smithsonian's Museum of History and Technology, with Fermilab as one of the centre-pieces).

This position is likely to be reflected in the proposed science budget for 1979 which President Carter will offer to Congress next week. In this it seems likely that a major sum will be allocated for Isabelle under the Department of Energy's construction programme, but that the sum earmarked for Fermilab's energy doubler will not be large, and will come from the laboratory's operating budget.

Wilson reaches retirement age next year, and would naturally like to see the doubler well under way—if not finished—before he goes. If the administration's proposed budget does not provide the money for him to do it, then his last chance would rest with the appropriation and authorisation committees in Congress which could— if so persuaded—add an additional earmarked sum.

David Dickson

Mediterranean pollution: one man's optimism

THE 1978 Monaco meeting of the 17 Mediterranean countries involved in the 'Mediterranean Action Plan' to clean up pollution closed last Saturday to such headlines as 'Deadlock over Med' and 'Pollution talks go into stagnation'. But in Geneva, the headquarters of the United Nations Environment Project's 'Regional Seas' programme, one man remains unrepentantly optimistic: the director of the programme, and inspiration of the Mediterranean Action Plan, Dr Stjepan Keckes.

Dr Keckes (his name is pronounced 'Keshkesh') is almost literally the power behind the Mediterranean plan. A 46-year-old marine scientist, member of the Hungarian minority of Yugoslavia, he has in 4 years established a ring of 79 cooperating research centres (some of them in perpetually antagonistic states such as Greece and Turkey, Egypt and Israel) and produced a baseline study of Mediterranean pollution which all governments respect—no mean achievement.

And with his principal colleagues Patricia Bliss (legal adviser) and Mohammed Tangi (environmental management) Keckes has been responsible for a sequence of inter-governmental agreements on pollution which, despite Monaco, has been one of the jewels in the UN's crown. Next month the first of two protocols to control dumping and environmental accidents come into force, ratified by six Mediterranean states (Spain, France, Yugoslavia, Tunisia, Lebanon and Monaco). Few international agreements move so fast: only 10% of the signatories to the 1973 London Dumping Convention, for

example, have ratified the Convention. Keckes can claim 33% in two years for the protocols of the 1976 Barcelona Convention on Mediterranean pollution. Keckes has a right to be optimistic.

Speaking this week from Geneva Keckes told *Nature* that he was "perfectly satisfied" with the meeting in Monaco, and that it had "fulfilled our expectations completely". That cannot be entirely accurate, however, for Keckes, interviewed by *Nature* in December, spoke of the Monaco meeting paving the way for the adoption of a third protocol—the one that bites, on land-based sources, which deliver 80% of the pollution load—"by the autumn" of 1978. At Monaco, however, there was little progress on the protocol and a further discussion session has been planned for Geneva in October—to be followed by a meeting in Athens to adopt the protocol at no specified date. Keckes, ever optimistic, is delighted that at least the place for the adoption of the protocol—Athens—has been decided.

The third protocol defines a 'black list' of substances that must on no account be released to the Mediterranean, and a 'grey list' which can be released but only in certain limited amounts. The governments involved have estimated that to implement the necessary controls would cost some \$10 billion in initial capital investment, a large part of it to control the industrial pollution flowing from the rivers Rhone (France) and Po (Italy). Difficulties have arisen over the North-South dialogue, with Southern Mediterranean states fearing that the con-



trols will inhibit their industrial development; and over whether the countries responsible for most of the pollution should pay compensation to the other states; and over the control of pollution in rivers.

Keckes remains satisfied that these difficulties will be solved, while recognising that agreeing the third protocol will be "the most difficult thing Mediterranean states will ever negotiate. We are not rushing them".

There were successes at Monaco. The most significant scientifically is the agreement to set up a study of, and consider a protocol on, air pollution over the Mediterranean. Air pollution will be "one of the biggest surprises in the future" Keckes believes. Much of the mercury and lead pollution arriving in the Mediterranean may travel first through the air, not water. And Keckes can now begin setting up some 10 to 15 new laboratories to monitor the air transport of pollutants. He expects results in 4 to 5 years. Another Monaco success for Keckes is his successful resistance of moves to make the pollution research reports secret. Keckes insisted they will be published; and they will be.

Keckes has ensured that governments approve their own research stations, so their results cannot be dismissed; and has insisted that the research be done by them rather than for them, to build up a national base for pollution monitoring. (UNEP has provided \$1.7 million for this purpose.) He has made sure that all results are intercalibrated, to be strictly comparable. And no doubt to the comfort of the least developed nations of the Mediterranean he also argues that for UNEP "protection of the environment is just one dimension of the development of the environment—for us protection for its own sake doesn't exist."

Robert Walgate



Stjepan Keckes: Confronting governments with reality

Rogers suggests interim legislation on DNA research

THE draft of a new bill covering both public and private research on recombinant DNA has been produced by Representative Paul G. Rogers in an attempt to break the deadlock on congressional legislation that developed late last autumn.

According to the provisions of the proposed bill, interim legislation would come into effect for a period of two years under which all such research would be required to be covered by guidelines laid down by the National Institutes of Health.

In addition, a commission for the study of research and technology would be set up charged with conducting a study of federal policy regarding activities involving the genetic modification of organisms and viruses. The commission would make a report within two years containing recommendations regarding federal action to be taken to promote, regulate or review such activities.

Mr Rogers is chairman of the sub-committee on health and the environment of the House Committee on Interstate and Foreign Commerce. Discussion on an earlier bill supported by the sub-committee was not completed by the full committee in the last legislative session, and the delay meant that the bill did not reach the floor of the house.

Under the provisions of the new bill, all those in the process of carrying out experiments involving recombinant DNA or proposing to do so would be required to provide an assurance to the secretary of the Department of Health, Education and Welfare that the work was being carried out in accordance with the NIH guidelines (the present guidelines, which were introduced in 1976, are currently being revised).

Those found to be ignoring the guidelines would be liable to a civil penalty (a fine) and the possible suspension of research funds.

Supporters of the new bill are hoping that by avoiding some of the more controversial aspects of the present debate—such as the problem of the 'prior disclosure' of research results, or the legal protection of employees who provide evidence of infringements—it will bring together house, senate and administration thinking, and have a higher chance of success than last autumn's attempt.

A further controversial issue is whether the federal government should be allowed to pre-empt local legislation. The bill's final position on this is likely to be the outcome of lengthy discussion with members of Congress and the administration.

But even if this approach fails to break the current deadlock, there is still a chance that the administration could develop a regulatory framework for both publicly and privately funded recombinant DNA research through existing legislation, namely section 361 of the Public Health Act.

Although the government had previously stated, following a petition from the Environmental Protection Fund in 1976, that it felt this legislation did not cover DNA research, recent re-examination of previous issues to which the act has been applied has indicated that this may indeed be possible.

Speaking at hearings held in

Washington last November by Senator Adlai Stevenson, Dr Gilbert Omenn of the President's Office of Science and Technology Policy said that the administration had rejected section 361 as being neither appropriate nor sufficient for recombinant DNA research.

However after looking at some of the previous applications more closely, the administration is beginning to have second thoughts, and is now looking closely at ways in which, if congress fails to come up with the appropriate legislation, regulations covering research with recombinant DNA could be implemented under section 361.

David Dickson

Tsiolkovskii's dream

THE link-up of Salyut-6 and Soyuz-26 and 27 marks a further step towards Tsiolkovskii's dream of a permanent orbital space station. Yet, rather strangely, the link-up was not used to effect a change of personnel, but merely to simulate one; the second crew—Vladimir Dzhanibekov and Oleg Makarov—returning to earth only five days after launch, and leaving the veterans—Yurii Romanenko and Georgii Grechko—still in orbit.

Salyut-6, with its two docking bays, represents a new generation of Soviet spacecraft. The docking bays are interchangeable, so that when Soyuz-25 failed to dock and it was suspected that there was some fault in the docking equipment, Soyuz-26, as back-up mission, docked in the second bay. Only after Romanenko and Grechko had checked out the first bay was this used for the linkup of Soyuz-27. Although there was no actual change of crew, the returning cosmonauts did change spacecraft, returning to earth aboard Soyuz-26. The launch of the second crew one month to the day after the first may be some indication of the shift-length planned for a fully operational space station; such a station, it is stressed, could save considerably on the time at present spent in warming up and mothballing the station at the beginning and end of singleton missions.

In addition to comforts for the "resident" crew—books, mail, newspapers—and the cherry juice required for the ritual toast—the "relieving" Soyuz-27 carried two temperature-stable "Biotech-8" containers containing, respectively, the paramecium and proteus cultures for the Franco-Soviet "Cytos" experiment, which studies the effect of space-flight on cell-division. The "visiting" crew also

carried out a study of blood circulation, data from which, it is hoped, can be used to develop prophylactic measures to help cosmonauts adapt to weightlessness. Dzhanibekov, an expert on radioelectronics, checked out the various on-board systems, and all four took part in the mechanical "resonance" experiment to determine the exact strength characteristic of the compound structure.

With mail delivered again to orbit (the first time since the Soyuz 4/5 linkup of 1969), a radiorepair man on call, and (according to veteran cosmonaut Valerii Kubasov) plans for refuelling the correction engines already under discussion, Tsiolkovskii's vision seems perceptibly nearer. Yet in one respect, Salyut-6 would seem to represent a retrograde step. Although, according to Grechko, the work aboard Salyut-6 is more interesting than his previous mission on Salyut-4, since the cosmonauts can now go for spacewalks, nevertheless he misses the "oasis, where we grow plants and peas" of the earlier craft.

Vera Rich

US industry attacks Ames test

SHORT-TERM bacterial tests for potential carcinogens, such as that developed by Dr Bruce Ames at the University of California, do not predict similar responses in humans, according to the American Industrial Health Council, a New York based lobby group for the US chemical industry.

In comments on legislation covering the industrial use of potential and known carcinogens proposed by the Occupational Safety and Health Administration, the council, whose members include representatives of the

major US chemical companies, says that while such tests have value as screening tools, they are too unreliable as predictors of human responses to be sufficient to warrant regulatory action.

The council recommends that emphasis should be placed both on data from animals exposed to low levels of potential carcinogens over normal lifespans, and where prior worker exposure has occurred, on human epidemiological data. It rejects the notion that it is realistic to impose a "no risk" approach to potential carcinogens, and suggests rather the development of

dose-risk data, using risk/benefit analysis to determine acceptable-risk levels of exposure.

The council also suggests that the National Academy of Sciences should set up a classification panel to examine the hazards of potential and known carcinogens, which would be responsible both for proper category assignments and for the modification of categories as relevant advances in science dictate.

AIHC believes that setting up such a panel would provide some degree of insulation from a wide variety of

"political and other pressures" to which regulatory agencies are subject, requiring such agencies to make politically-acceptable decisions and to pay "somewhat less attention" to their scientific basis.

"The classification of carcinogens is a scientific, not a regulatory question, and would be much better handled by an independent group of scientific people than by a regulatory agency" according to Dr Ellwood P. Blanchard of the Dupont Company, a member of the AIHC steering committee.

David Dickson

Dioxin meeting recommends cancer study

SINCE the release of dioxin (the isomer 2, 3, 7, 8 tetrachloro dibenzo-p-dioxin) over a populated area at Seveso in Italy on 10 July 1976, there has been fear that the chemical is a carcinogen. Few data have been available, but it is beginning to seem that the fear is justified; hence the meeting in Lyons last week hosted by the International Agency for Research in Cancer (IARC) and the US National Institute of Environmental Health Sciences. The conclusion of the meeting was that a major epidemiological survey should be established.

It is now known that there have been at least 14 incidents in different chemical plants throughout the world where workers have been exposed to chlorinated dibenzo dioxin. In two cases the public has been exposed: at Seveso and in the herbicide spraying programme in Vietnam. In the latter cases large populations were exposed to low levels of dioxin; but while these populations must be monitored to assess the public health risk associated with low level exposure, at Lyons the opinion was that little could be learned from them. If dioxin exposure was to be unambiguously related to clinical and pathological findings, the meeting argued, the chemical plant workers were the group to monitor.

Three of the industrial accidents discussed in Lyons occurred between 20 and 30 years ago. Evidence is available from one of these accidents to suggest that in recent years there has been a marked increase in certain types of carcinoma in workers exposed to dioxins.

However the importance of this finding is marred by the small numbers of workers monitored. The meeting considered that it was therefore an urgent matter for a larger population to be studied to detect trends in carcinoma incidence which would be statistically significant. It urged the

chemical companies concerned to make their records available for scrutiny. And the meeting stated in no uncertain terms that all the workers exposed to dioxin—whether they are still employed by companies or employed elsewhere—must be located. Mortality trends for this population must be collected.

Two groups reported animal studies to test the carcinogenicity of dioxin: the first directed by Dr James Allen at the University of Wisconsin, and the second by the Doll Chemical Company. Both research teams identified the dioxin as a carcinogen. But the Wisconsin team claims that the chemical is carcinogenic at a concentration 700 times lower than the level that produce tumours in Doll's study. Reasons for the discrepancy are still not clear, but it was pointed out at the meeting that the Wisconsin study is based on an extremely low number of animals.

Some of the companies involved in dioxin accidents fear further independent investigation, not wishing to run the risk of vast compensation claims. And the meeting felt that, if companies made it a condition that their participation in a dioxin workers' survey were to be treated in confidence, this wish must be respected. The view was that, wherever the blame lies, the most important task at the moment is the compilation of information; and this the IARC is willing to undertake. Dr Rodolfo Saracci of the Unit of Immunology and Bio-Statistics agreed to direct some of this work.

The IARC will act as a clearing house for data on chlorinated dibenzo dioxin—with the help of a permanent secretariat co-opted from participants at the meeting. One of its first tasks will be the production of a list of recommended clinical procedures for examining people exposed to dioxin. It will be stressed that where possible the

clinical findings should be related to body dioxin levels—the 'body burden'. This information together with the mortality data should leave the IARC better able to assess the danger to health caused by exposure to dioxins; cancer may be only one of the possible risks.

Alistair Hay

NASA chooses its space lab candidates

FOLLOWING a similar announcement from the European Space Agency (*Nature*, January 5), the US National Aeronautics and Space Administration has announced the six American scientists from whom one will be selected as a payload specialist on the first flight of Spacelab, due to be flown in NASA's Space Shuttle in 1980.

The names of the finalists are:

Dr Craig L. Fischer (40), of the Palm Desert Medical Group Inc, California; Dr Michael L. Lampton (36), of the University of California, Berkeley; Byron K. Lichtenberg (29), Massachusetts Institute of Technology; Dr Robert T. Menzies (34), NASA Jet Propulsion Laboratory, Pasadena, California; Ann F. Whittaker (38), NASA, Marshall Space Flight Center, Huntsville; and Dr Richard J. Terrile (26), California Institute of Technology, Pasadena.

Five of the ten candidates that have now been named by NASA and ESA will undergo extensive training following a final selection in early spring. Of these, two will go into space, while the other three will perform support and advisory roles in the control centre on earth. □

Government accuses nuclear industry of using propaganda

THE US Government has accused its industrial partner in its fast breeder reactor development project at Clinch River, Tennessee, of producing pam-

phlets that give an "oversimplified" and "distorted" view of the dangers of nuclear power.

In a report published in Washington last week, the General Accounting Office said that the Breeder Reaction Corporation of Oak Ridge, Tennessee, which represents almost 700 companies involved in the fast breeder programme, had produced a series of thirteen pamphlets about the programme, at least four of which "clearly constitute propaganda and as such are questionable for dissemination to the public".

In particular, the GAO questioned the statement in one pamphlet that plutonium is "not a realistic threat when compared with other hazardous materials". It says that the pamphlet grossly understates the dangers, and does not inform readers that plutonium is extremely toxic, and in some circumstances carcinogenic. □

US ban use of prisoners in drug research

THE US Department of Health, Education and Welfare is planning to ban the use of prisoners for drug research not directly related to their health or well-being.

This suggestion follows last year's report of the Commission for the Protection of Human Subjects of Biomedical and Behavioural Research, suggesting stringent conditions on such research to ensure that the participation of prisoners was voluntary.

In its response to comments on the Commission's report, published last week (January 6), the department says that the Commission found "a paucity of evidence" that research testing of drugs involving minimal risk on prisoners was necessary (see p. 197).

In issuing proposed regulations on research involving prisoners, the department has therefore suggested that the use of prisoners for biomedical and behavioural research studies other than those relating directly to the causes and conditions of their imprisonment, and their well-being in prison, should be banned.

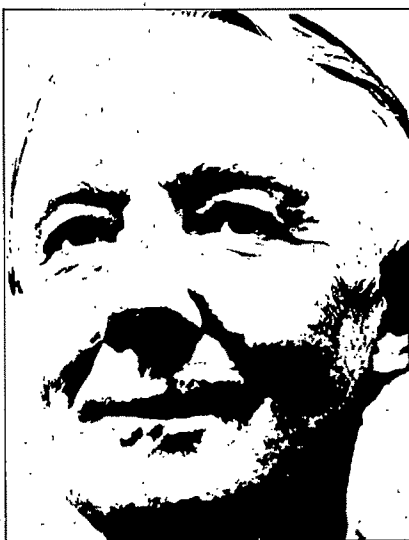
"The Department has concluded that the need to assure that research on human individuals is performed only on human subjects is performed only on individuals who have knowingly and voluntarily consented to participate far outweighs any need that has been shown for the use of prisoners in these studies" it says.

David Dickson

THE high rate of rickets in children of Asian origin in Britain is causing concern, and a demand that the government "does something about it". One suggestion is that various foods including the flour used to make chapatis, should be fortified with vitamin D. This proposal is being resisted by some scientists, because the present addition of calcium and vitamins to bread, and of vitamins to margarine, is now under attack. It is pointed out that there is little or no evidence that this costly and complicated scheme is having any beneficial effect on the nutritional standards of the population, and that more experimental evidence should be sought. It is probable that if every Asian child in Britain took a spoonful of codliver oil daily, rickets would disappear among this section of our population. Efforts to ensure that the vitamin went where it was needed would be more effective than giving everyone unnecessary and unwanted supplements.

Those who favour more mass fortification of food have the mistaken belief that as we all need small amounts of substances like vitamin D to preserve our health, then the more we consume of them the healthier we will become. This false opinion is perpetuated by many advertisements. I have before me one which tells me this preparation is rich in protein, iron and vitamins B1 and B2. It goes on to say that protein is essential for growth and bodily repairs, iron to nourish the blood, and the vitamins to aid digestion, convert food to energy and to maintain tissues such as nerves, muscles and the skin, and generally to promote "vitality". Seed

Enough is enough



KENNETH MELLANBY

merchants tell us that the more expensive varieties of vegetables give plants particularly full of protein, and with extra amounts of vitamin C and minerals. Once again they claim that these substances must be taken if we are to remain healthy.

All these statements about the need to eat protein and vitamins are of course true, and if we were living on deficient diets the advertised commodities would no doubt do us good. But when our diet contains the required minimum of these substances, and addition is generally useless and often harmful.

Unfortunately in Britain it is often the better educated who talk the most

nonsense about diet. They have learned some of the elements of nutrition and know all about deficiency diseases. They seldom realise that most of our over-fed adults have a surplus of protein and other essentials, and that most of the allegedly health-giving supplements would be best flushed down the drain.

Yet malnutrition, in various forms, is common. In all Western countries the most common and obvious form is gluttony with consequent obesity. Those who try to slim by eating less are subject to a barrage of advertisements for low-calorie foods containing large amounts of supplements. These are generally unnecessary, for the slimmer lives on a nutritious diet of (his own) human flesh. Then the appalling state of our teeth is a symptom of some dietary imbalance, partly but not entirely connected with our overconsumption of sugar. But on the whole most adults seem not to be short of protein and vitamins, so more of these are not "good for them". The only common deficiency would seem to be fibre, and there is evidence to suggest that added bran does some good, but most supplements seem to be useless or harmful.

The position with young children and the elderly is rather different. These groups have a limited food intake, and in Britain today up to half of their calories may come from sugar, in sweetmeats for children, in hot sweet tea for the old. This leaves little room for essential nutrients, so it may be necessary to add these in concentrated form. But it would be more sensible to cut down on the sugar, which, in excess, is always harmful.

correspondence

Soviet genetics

SIR,—Dr Italo Barrai in his letter (*Nature* 271, 8; 1978) commenting on my earlier article on the new controversy on human genetics in the USSR (*Nature* 268, 285; 1977) asks me two questions which I shall answer here.

Barrai questions my ability to describe the situation in genetics "... what can a gerontologist say about human genetics: in what position is he to pass value judgement?"

It is true that most of my research while in the USSR and here in UK had been related to problems of ageing. However, my book *The Rise and Fall of T. D. Lysenko*, published by Columbia University Press in 1969 is still the only book written and published by a Russian scientist about the history of genetic controversy in the Soviet Union. This book also described the history of medical genetics in the USSR from 1920 to 1967. My article in *Nature* was in some way a continuation of these accounts.

Barrai's second question asks that I prove that in 1976 N. V. Tsitsin was not yet appointed president of the Genetic Congress (which takes place in Moscow in August 1978). The proof comes in the decision about the Moscow Congress made at the previous Congress in Berkeley in 1973. At this Congress D. K. Beliaev, in his capacity of the President of the Soviet Genetic Society, made an invitation on behalf of the Government of the USSR, to hold the next Congress in Moscow. As soon as the invitation was accepted D. K. Beliaev started to make all necessary arrangements as acting President. N. V. Tsitsin's name as president of the Congress appeared only in 1977, and I believe that it surprised not only me, but also the International Genetic Federation. N. V. Tsitsin, now 80 years old, was never very popular among geneticists, mostly because the way in which he acquired prominence in the mid-1930s. One can read about this in the book by Prof. D. Joravsky *The Lysenko Affair* (Harvard University Press: Boston, 1970)—

"In 1932 Tsitsin moved to the West Siberian Experimental Station in Omsk, whose director, V. R. Berg, believed in the practicality of crossing wheat with couch grass. He had been

working on the problem himself.

"A year after Tsitsin's arrival in Omsk, Berg was arrested as a 'wrecker', and Tsitsin was made a hero by the mass media, even though (or because) he still complained that 'an enormous portion of specialists up to the present are extremely negatively inclined toward our work.' A commission of inquiry from the Commissariat of Agriculture, which was itself being purged of 'wreckers', decided that Tsitsin's work had enormous promise and merited great support. He was given space in *Pravda* to promise an annual hybrid of wheat and couch grass ready for production testing by the fall of 1935; a perennial would take a year longer. When 1935 came to an end without a hybrid ready for the testing service, Tsitsin received reassurance from the highest authority. Stalin told him, and allowed the awesome words to be inscribed on newsprint: 'Experiment more boldly. We will support you'. Tsitsin became the director of the Omsk station, which had been promoted into the Siberian Institute of Grain Culture. Specialists began to pay respectful attention to his work."

Everything in this description is correct. One can find details of the events in *Pravda* for 9 July 1934 and 30 December 1935, pp. 81–82. N. V. Tsitsin has still not made perennial wheat, but as a director of a big plant breeding research institute after the second world war he could be given credit for some achievements in wheat breeding. He has not played any serious role in Soviet genetics for the last five years; and his name was removed from the editorial board of the Soviet journal *Genetika* at the very beginning of 1973. If Soviet geneticists had had a free choice of who was to be president of the first International Congress on Genetics held in the USSR I doubt very much that Tsitsin would have got the job.

Dr Barrai also asked me to give a fuller justification of my negative assessment of N. P. Dubinin's current work. I am afraid that this needs more space than this letter permits. Dr Barrai, however, partly provided the answer himself. Trying to explain some false methods in N. P. Dubinin's publications Dr Barrai wrote "... it might well be that, as director, he signs work from his institute which might be

beyond the capacity of his technical judgement". Some of my colleagues in the USSR also thought that this could be the case. They thought, however, that this could equally be said about publications which were not failures. In any case the Moscow Congress will provide enough information about the situation in Soviet genetics today for those who do not restrict their interest to the official programme.

Yours faithfully,

ZHORES A. MEDVEDEV

National Institute for
Medical Research, London

NIH guideline ineffective

SIR,—The National Institute of Health *Guidelines for Recombinant DNA Research* recommended 2% aqueous Wescodyne, an iodophore that is used in many hospitals and laboratories as a disinfectant, as a decontaminant for biological safety cabinets and 5% for a spill outside a cabinet. A contact time of 10 to 15 minutes was given for the 2% solution and 20 minutes was considered adequate for the 5% concentration.

However I have conducted experiments which indicate:

- Aqueous Wescodyne (5%) is ineffective when used for 80 minutes against poliovirus in a test mixture containing 8.5% bovine serum albumin (a mixture equivalent in protein concentration to the higher range in serum).
- Wescodyne (10%) employed under the same conditions for 40 minutes is also ineffective.
- Wescodyne (10% v/v) in 50% ethanol (w/w) was effective and this mixture, originally recommended for hand washing, should be considered for use in biohazard situations, particularly for decontamination of work surfaces and biological safety cabinets.

These results are significant, for if a virucide cannot inactivate poliovirus one would be concerned about using the virucide against hepatitis B or SV40 viruses.

Yours faithfully,

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news and views

Pseudogene structure in 5S RNA genes

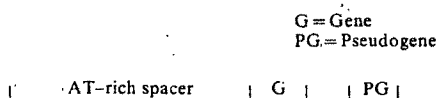
from Peter Ford

THE 5S RNA genes of *Xenopus laevis* have several obvious advantages as a system for studying specific gene regulation in higher eukaryotes. The 5S RNA itself is a small (120 nucleotides) readily purified molecule which is easy to identify by simple sequencing techniques. As a component of ribosomes it is found throughout the living world and has an essential though poorly understood function in protein synthesis. It is synthesised in its final form with no obligatory processing at either 5' or 3' terminus unlike most other RNA species, which means that correct termination and more important correct initiation of transcription may be easily assayed. In addition *Xenopus laevis* expresses different sets of 5S RNA genes in somatic cells and oocytes, a property which is phylogenetically widespread since it also occurs in other amphibians and teleost fish, and implies a general mechanism by which differential 5S gene expression is controlled. No one understands at present the functional significance, if any, of different 5S RNA sequence variants in oocytes and somatic cells, and for the purposes of understanding gene regulation this is not important.

D. Brown and his collaborators reported the purification of 5S DNA from *Xenopus laevis* a number of years ago (Brown, Wensink & Jordan *Proc. natn. Acad. Sci. U.S.A.* **68**, 3175; 1971). The genes are highly repeated (24,000 per haploid genome) and tandemly linked into several clusters (perhaps as many as 18) at the ends (telomeres) of chromosomes (Pardue, Brown & Birnstiel *Chromosoma* **42**, 191; 1973). As it turned out the purified 5S DNA prepared by Brown is of the oocyte type, the somatic type has not so far been isolated from *Xenopus laevis*, which means that the work done so far on purified 5S DNA relates only to the oocyte type sequences. The bulk of the

purified 5S DNA has a regular alternation of AT-rich with GC-rich sequence within each repeating unit which is about 700 base pairs long. Although different repeating units are very similar to each other they may differ in length and in sequence detail. Length heterogeneity has a quantal element resulting from a variable number of a 15 base pair mini repeat located in the AT-rich region but is complicated by presence or absence of other specific AT-rich sequences. The GC-rich region which contains a single 5S RNA gene is very constant in length (350 base pairs) but may vary in sequence detail.

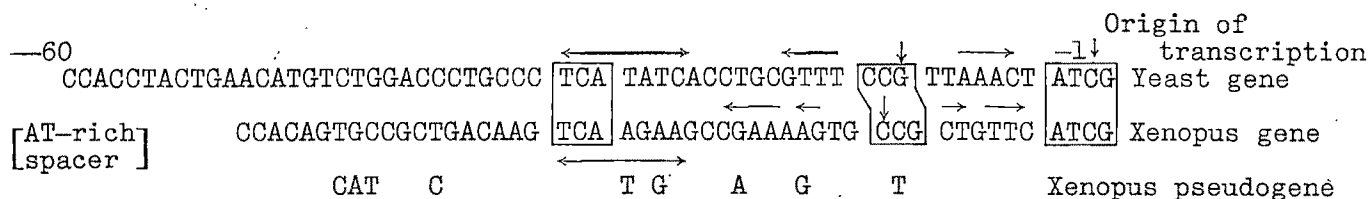
What has come as a surprise to many people is the recent demonstration by two independent sequencing techniques (Jacq, Miller & Brownlee *Cell* **12**, 109; 1977; Fedoroff & Brown *Cold Spring Harbor Symp. quant. Biol.* **42**, in the press) that the GC-rich region of 5S DNA also contains a pseudogene which is identical to the first 101 base pairs of the gene at all but 10 sites. The pseudogene completely lacks the last 19 base pairs of the gene. The pseudogene is detected in both total genomic 5S DNA as well as in purified individual repeat units cloned in bacterial plasmid vectors and can thus be assumed to occur in the majority if not all of the repeat units in oocyte type 5S DNA. A map of the repeat unit now looks like this



Attempts to show that the pseudogene is transcribed *in vivo* have not been successful suggesting that pseudogenes are not transcribed or that their transcription products are very short lived. Until positive evidence for pseudogene transcription is found it seems reasonable to assume that they are inactive. Now both the gene and

the pseudogene are preceded by GC-rich sequences 49 base pairs long which are identical in all but nine positions. By analogy with prokaryote systems the region preceding a gene is expected to contain promoter sites for RNA polymerase binding and perhaps regulatory sites for the control of transcription. Since all the information required for the correct initiation and faithful transcription of the 5S gene is present within one repeat unit as was shown by the successful and faithful transcription of cloned single repeat units after microinjection into *Xenopus* oocyte nuclei (Brown & Gurdon *Proc. natn. Acad. Sci. U.S.A.* **74**, 2064; 1977; Fedoroff & Brown *op. cit.*), it seems reasonable to conclude that the specific absence of pseudogene transcription is due to the absence of the information required for initiation of transcription in the pseudogene. This means that either the information for correct polymerase binding resides in the AT-rich region which comes before the GC-rich 'promoter' region of the gene or it has been specifically destroyed by some or all of the nine base changes which differentiate the 'promoter' region of the pseudogene.

The sequence for a 5S gene region from the budding yeast, *Saccharomyces cerevisiae*, has been determined recently (Valenzuela *et al.* *Nature* **267**, 641; 1977; Maxam *et al.* *Nature* **267**, 643; 1977) and it may be instructive to compare the 'promoter' region with that found in *Xenopus*. The rationale for this comparison lies with the observation that prokaryote promoters show AT-rich regions which by mutation analysis are known to be involved in polymerase binding. One site which allows initial recognition and entry of the polymerase is located about 30–35 nucleotide pairs upstream from the origin of transcription. A second AT-rich site, the tight binding site, is located 10 nucleotide pairs from the origin of transcription. There is some but not a great deal of sequence con-



servation at these sites when different promoters are compared. Comparison of the yeast and *Xenopus* 'promoters' shows very little sequence similarity as expected, but there are two structural features in common and only future work will show if they are significant. A sequence with twofold rotational symmetry is centred 11 (yeast) and 12 (*Xenopus*) base pairs from the origin of transcription and contains a CCG sequence off centre. The second feature is the presence of an AT-rich region seven nucleotides long which has a trinucleotide in common and is centred 27 base pairs away from the origin of transcription. Both these sequences are mutated in the pseudogene 'promoter' consistent with the notion that they are important in the normal faithful origin of transcription.

The presence of an inactive pseudogene presents an evolutionary paradox. Since it is not transcribed it is prob-

ably non-functional although it may have some other unknown function for which it has been selected and preserved in evolution (Jacq *et al. op. cit.*), but the mutations which make it non-functional are present in every copy and must have arisen once in the original pseudogene. The alternative that the same mutations have occurred many times at the same sites in different pseudogenes is not only unattractive but a highly improbable set of events. As it now exists the pseudogene sequence would produce a partial 5S molecule which might compete with the normal product for ribosome binding but render such ribosomes inactive or inefficient in protein synthesis. There would be heavy selection against such a gene activity and mutations would rapidly accumulate to inactivate or eliminate the pseudogene. The evolutionary paradox is thus seen as a question how can a functionless

potentially deleterious sequence spread in a genome to the extent that the 5S pseudogene has spread as if selected for some particular purpose? Could it be as Fedoroff and Brown suggest that the events leading to the spread of the gene itself have also led to the spread of the pseudogene as an unwitting 'fellow traveller of an evolutionarily successful gene'?

Whatever the actual evolutionary history of the pseudogene it is clear that future work will establish the regions essential for polymerase binding and possibly for control elements in 5S DNA. Such experiments will make use of the *Xenopus* oocyte nucleus as a vehicle for testing mutated and mutilated cloned 5S DNA repeats. What of tissue specific control? It would be useful to have the somatic type 5S DNA cloned and purified for comparison but that is another story and must wait for another day. □

Rhizobium recognition

from John E. Beringer

ALL *Rhizobium* species have the capability to form nitrogen-fixing nodules on the roots of leguminous plants. However, not all legumes are nodulated and those that are usually interact only with a particular 'species' of *Rhizobium*. This host-range specificity has long been an intriguing problem but, until recently, little was known about its biochemical basis. An understanding of the mechanisms should not only enhance our knowledge of the symbiosis, but also indicate ways in which *Rhizobium* and plant species may be manipulated genetically to increase the range of nodulated plants.

It is thought that host range is determined before the bacteria penetrate the surface layers of the root and that it is based on the specific recognition of the 'correct' *Rhizobium* species by the plant in a manner analogous to antigen-antibody binding. What makes this an attractive model for the host-range interaction is the observation that plants produce compounds called lectins that are able to bind to 'antigens' and are known to play a part in the plant's defence mechanism against pathogenic microorganisms.

The lipopolysaccharides of Gram-negative bacteria are well known antigens, primarily because they include the O antigens of *Salmonella* species. The rhizobia are Gram-negative bacteria and share the same general pattern of surface structure as the salmonellae. Working outward from the cytoplasm this includes a mucopeptide layer, then lipopolysaccharide, often a polysaccharide capsule and sometimes a further, less adherent, layer of structurally fairly simple exopolysaccharide. Because lipopolysaccharides are known antigens it might be expected that if there are host-specific antigens recognised by legumes they would reside in this layer of the cell wall.

There have been a number of reports in the literature which indicate that lectin binding by *Rhizobium* species may be involved in the host-symbiont recognition process. Dazzo and Hubbell (*Appl. Microbiol.* 30, 1017; 1975) have even produced a model for the recognition process based on the observation that a common antigen occurs on the surfaces of the roots of clover and on nodulating strains of *R. trifolii*. They suggested that the binding of multivalent lectins to these common antigens could be the method whereby the cor-

rect host and *Rhizobium* species were brought into close contact, allowing the interaction to proceed to a point where penetration could occur. Despite anomalies that have been reported in lectin-*Rhizobium* binding studies, it is now generally accepted that recognition is likely to involve lectin binding.

Having shown that lectin binding was important, an obvious step was to determine the nature of the bacterial antigen(s). Dazzo and Hubbell suggested that the antigenic determinant(s) of *R. trifolii* strains resided in the capsular polysaccharide and later Dazzo and Brill (*Appl. environ. Microbiol.* 33, 132; 1977) reported that this binding was mediated through 2-deoxyglucose-sensitive binding sites present on clover root hairs and in the capsular polysaccharide of nodulating *R. trifolii* strains. They also isolated the binding protein (lectin) of clover which bound to *R. trifolii* but not to two species which do not nodulate clover, *R. meliloti* and *R. japonicum*. This attractive piece of research served to indicate that at least the initial stages of host-symbiont recognition were simply based on the presence of a common receptor in the surface layers of the host and bacterium. However, the situation is not so clear because Wolpert and

Albersheim (*Biochem. biophys. Res. Commun.* **70**, 729; 1976) have reported that legume lectins interact with the lipopolysaccharide of their 'correct' *Rhizobium* species.

A classic way to examine the importance of cell wall components for antigenicity or phage attachment has been to examine mutants defective in their synthesis. This approach has been taken by Sanders, Carlson and Albersheim who report their findings with *R. leguminosarum* in this issue of *Nature* (page 240). By selecting bacteria after passing cultures through 1.2 μ m Millipore filters they obtained mutants at high frequency which did not produce exopolysaccharide. Chemical characterisation of the mutants indicated that there was no exopolysaccharide synthesised and that the capsule and lipopolysaccharide were unaltered. Five of these mutants, otherwise apparently indistinguishable from the parent, were tested for their ability to nodulate their correct host plant, the pea; all five mutants were non-nodulating. Five spontaneous revertants of one of these mutants, selected for the reappearance of exopolysaccharide production, all regained the ability to nodulate the pea. Thus it appears that the exopolysaccharide is essential for nodulation to occur, at least in this strain of *R. leguminosarum*. Whether or not it is involved in lectin binding has not yet been determined.

It now remains for these and other workers to clarify the rôle of capsular, lipo- and exopolysaccharides in determining the host range of *Rhizobium* species. Indeed it has yet to be shown how complex the host recognition process is. Perhaps lectin binding is only one of a complex series of recognition processes that must occur before a nitrogen fixing nodule can develop. □

Mining triggers earthquakes

from Peter J. Smith

It is almost universally accepted now that, given the right circumstances, earthquakes may be induced artificially by injecting fluids into the ground, by setting of underground nuclear explosions or by filling large reservoirs. Indeed, the idea is so commonplace that there may be some difficulty in believing that less than 7 years ago it was the subject of lively controversy, largely because the evidence in favour of man-made earthquakes, though strong, was then little more than cir-

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Animal sounds: coloratura and basso profundo

from John Krebs

MANY species of birds and mammals use sounds as threat signals in contests over territory and other resources. Dogs growl, elephants roar, and great tits scold. The remarkable thing is that the threat sounds of most birds and mammals tend to be low-pitched, harsh (broad frequency spectrum) noises. In contrast non-aggressive signals, such as appeasement gestures, tend to be high pitched and contain pure tones. E. S. Morton (*Am. Nat.* **111**, 855; 1977) now suggests an explanation.

Threat signals in the animal world to some extent replace out-and-out fighting, presumably because it is less dangerous for an individual to settle a dispute in this way. But obviously the loser of the contest, which might be for example over a piece of food, does less well than the winner and so one might expect, anthropomorphically speaking, losers not to give up easily when faced with a threat signal unless they are sure that they would lose eventually. The inescapable conclusion is that at the beginning of a contest animals try to assess the fighting ability of their opponent without actually engaging in a scrap. What sort of cues therefore would give reliable information about an opponent's fighting ability? A totally arbitrary cue would be easily faked, but some body features are inevitably correlated with strength, an obvious example being body size.

The significance of low pitched threat sounds should now be apparent: on the whole, larger individuals are capable of producing lower pitched sounds. The pitch of a threat call depends on the tension, size and thickness of the vibrating membrane on the voice box, and at least in mammals, on the volume of the nasal and oral cavities which act as resonators. In other words, depth of voice gives a quick, rough indication of how big an opponent is, and so it is not surprising that low pitched sounds have, during evolution, become an effective way of threatening a rival. Harshness is often an inevitable by-product of low frequency sounds, since a vibrating membrane under low tension will tend to produce harmonically

unrelated tones.

Why should appeasement and non-aggressive sounds be high pitched? One possibility is Darwin's Principle of Antithesis—signals conveying opposite kinds of information tend to be very different to reduce confusion—but Morton also suggests that high pitched appeasement calls have, so to speak, parasitised the fact that animals respond in a non-aggressive way to the high pitched begging calls of their young.

Coloratura, according to my dictionary, refers to 'incidental trills introduced to make a song or other piece of music more agreeable.' The song of our common or garden wren (known across the Atlantic as the winter wren) has enough coloratura to make Rossini's *Una voce poca fa* sound like a Gregorian Chant, but does this make it more agreeable to other wrens? D. Kroodsma (*Am. Nat.* **111**, 995; 1977), suggests that it does. He studied song recordings of the nine species of North American wren, which show a considerable range of complexity. Virtuosi such as the long-billed wren have over 100 different song types for each individual male, while the less ostentatious male Bewick's wren has between 9 and 20 song types. Kroodsma calculated for each of his nine species an index of song virtuosity, including information about the number of song types and the complexity of each type (for example our own wren has few song types but each one is very long and complex, so this species scores high on overall virtuosity.)

One conclusion of this analysis is that wren species with the most complicated songs are those species in which males are most polygynous. In polygynous species, the males compete intensely for females to add to their harems, and as the females have a choice of mate, they exert powerful sexual selection on males. Any male trait which increases his attractiveness to females will be favoured, and it seems that coloratura singing in wrens is just such a trait. It is an auditory peacock's tail.

John Krebs is a lecturer in Zoology at the Edward Grey Institute, University of Oxford.

cumstantial. What was lacking in most cases was a detailed record of seismic conditions for the period before the suspect events had become quite ob-

vious even to the least sensitive member of the population. In short, 'before and after' seismic comparisons were generally not available—a data gap

which led inevitably to a credibility gap.

Thanks largely to the controlled experiments at the Rangely oil field in Colorado (see for example Raleigh *et al. Science*, **191**, 1230; 1976) and the wider recognition of the role played by fluids in seismic processes generally, the reality of man-made earthquakes is no longer in doubt. Nevertheless, well-documented examples of the onset of such events are still quite rare, which makes the observations in western New York state by Fletcher and Sykes (*J. geophys. Res.* **82**, 3767; 1977) of more than routine interest. They also exemplify astonishing good luck. Fletcher and Sykes set up their seismometer array in 1970 in anticipation of the injection of fluids into a deep well at a steel plant just south of Buffalo. The planned injection never actually took place, but seismic activity increased anyway. What Fletcher and Sykes were unaware of at the time was that a hydraulic salt-mining operation was about to begin nearby at Dale, New York.

As it happens, the Attica-Dale area is already the most seismically active region of western New York state, but only in that there is a history of isolated, moderately large earthquakes. There have apparently been no swarms of smaller events in the recent past; and certainly none was recorded by Fletcher and Sykes from June 1970 to early August 1971, a period during which there was an average of only one seismic event a month. Unfortunately, the recording equipment was out of action for much of August, September and October 1971; but when normal service was resumed at the end of October, Fletcher and Sykes found they were observing microearthquakes at rates of up to 80 a day. The contrast with the year to August 1971 was startling.

The foci of these events were found to lie in the vicinity of a high-pressure injection well used in the hydraulic mining of salt. If Fletcher and Sykes had known of this operation in advance they would probably have taken greater care to have their recording apparatus in working order at the crucial time in early August 1971 when the well pressure was raised to its maximum of more than 60 bars. As it was, the onset of pressure-induced earthquakes went largely unrecorded. On the other hand, there is little doubt that the high activity observed from October onwards was the result of well fluid pressure, for when that pressure was allowed to fall suddenly to below 10 bars in mid-November the microearthquakes ceased equally spectacularly.

An important clue to the direct origin of the earthquakes comes from their distribution, for their foci lie not

only close to the injection well but also in an elongated zone along the Clarendon-Linden fault which passes within 50 m of the well bottom. The

obvious interpretation is that increase in pore pressure resulting from hydraulic mining triggered seismic activity in the Clarendon-Linden fault zone. □

Functions of the septo-hippocampal system

from A. H. Black

The Ciba Symposium on the Functions of the Septo-Hippocampal System was held in London on October 17-20, 1977. The proceedings will be published as Ciba Foundation Symposium 58 (Elsevier/Excerpta Medica/North Holland, Amsterdam and New York, 1978). The symposium was organised by Dr J. A. Gray and chaired by Dr L. Weiskrantz.

ALTHOUGH considerable attention was focused on the anatomy and physiology of the septo-hippocampal system, the symposium was primarily concerned with behavioural functions. Let me first point to a difference between the septum and hippocampus. Both H. Ursin (University of Bergen) and S. P. Grossman (University of Chicago), who described research into the effects of septal damage, argued that the septum was a complex structure involved in a number of different, perhaps unrelated, behavioural control circuits, and, therefore, that one could not ascribe a single or even a few functions to it. On the other hand, the discussion of the hippocampus (and those areas of the septum to which it was intimately connected) suggested that it might carry out a small number of related functions. I shall focus on the discussion of those functions.

There was little support for the hypothesis that the primary function of the hippocampus is to inhibit behaviour. This hypothesis dominated the non-human literature in the late 1960s and early 1970s. Its relatively sudden demise (perhaps only temporary) is all the more striking. Three other possible functions of the hippocampus received considerable attention. The first was the plasticity of the hippocampus and its possible role in learning. P. Andersen (University of Oslo) presented some elegant data on

the synaptic potentiation that follows repetitive stimulation in isolated transverse slices of hippocampal tissue. After short periods of tetanic stimulation of isolated input fibres to CA1 cells there was a long lasting (from 30 to 75 min) increase in the extracellular excitatory postsynaptic potential recorded from the apical dendrites. Also, single units showed a corresponding increase in their probability of firing. These changes were seen without any change in the size of the afferent fibre volley. These results indicated that the potentiation could be attributed to changes at the synapse—either to an increased amount of transmitter or to a changed postsynaptic resistance locally in the dendritic tree. Additional data on the plasticity of the hippocampus were provided by G. Lynch (University of California) from an experiment in the freely moving unanaesthetised rat. The rats were first trained to press a lever when a tone was sounded. During the course of such training an evoked potential recorded from the outer molecular layer of the dentate gyrus gradually developed to the tone. After training to a single tone had been completed, the rats were trained to discriminate between two tones. The evoked potential was now seen to both tones, and the tones elicited discharge of the granule cells even though they had not done so before. At first, fairly long bursts of single unit activity were observed to both tones. But, as time passed, long bursts of firing to the reinforced tone continued while the burst of firing to the non-reinforced tone decreased in duration. Lynch suggested that these results are consistent with the view that the tones produce an input to the hippocampus by way of the perforant path; the effects of the input increase gradually during the early stages of learning—perhaps by the mechanism described by Andersen above, and earlier by Bliss and Lomo. Whether this input results in cellular discharge depends, however,

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MANY plants, particularly aquatic ones, experience periodic or even permanent waterlogging in their environment. The selective advantages conferred by morphological adaptation to such conditions is evidenced by the widespread occurrence of lacunae and large intercellular spaces within aquatic species. It has been demonstrated that oxygen penetrates along such interconnecting channels, reaching the root or rhizome extremities. For example, Coult and Vallance (*J. exp. Bot.* **9**, 384; 1958) showed that oxygen moved the full length of a 45-cm rhizome of *Menyanthes trifoliata* when it was suspended in an oxygen-free atmosphere but with the leafy shoot exposed to air.

Individual cells, nevertheless, may find themselves in conditions of low oxygen tension. Under such conditions, metabolic adaptations are essential, particularly the avoidance of ethanol accumulation as a result of anaerobic glycolysis. McManmon and Crawford (*New Phytol.* **70**, 299; 1971) examined a range of flood-tolerant and flood-intolerant species under waterlogged conditions and they found that some species, such as *Pisum sativum* and *Vicia faba* which are flood-intolerant, showed greatly increased levels of alcohol dehydrogenase (ARH, involved in acetaldehyde to ethanol conversion) under flood conditions and also high activity of 'malic' enzyme which converts malate to pyruvate (which can then be converted to acetaldehyde and hence to ethanol). Flood-tolerant species, such as *Phalaris arundinacea* and *Glyceria maxima* were found to decrease in their ADH activities as a response to flooding and showed no activity of 'malic' enzyme.

McManmon and Crawford proposed that flood-tolerant species lack malic

Adaptations to waterlogged environments

from Peter D. Moore

enzyme so that a diversion of glycolysis to malate production does not result in further malate decarboxylation to rejoin the path of anaerobic glycolysis and ethanol production, but ends in malate accumulation. Malate build-up seems to be harmless in these cells. In the flood-intolerant species any malate produced is decarboxylated by 'malic' enzyme, ADH is induced and ethanol accumulates to the detriment of the cell membranes.

Crawford and Bains (*New Phytol.* **79**, 519; 1977) have recently demonstrated that the build-up of ethanol in the roots of forest trees on waterlogging varies from one species to another. Thus, in the Sitka spruce (*Picea sitchensis*) which is intolerant of flooding, ethanol concentration had increased to 12 times its original level (to 5.6 $\mu\text{mol per g}$ fresh weight) after 24 h in anaerobic conditions, whereas in *Pinus contorta*, a flood-tolerant species, ethanol accumulation increased only three times its base level (0.7 $\mu\text{mol per g}$ fresh weight). Once again, tolerance to flooding is associated with a control of ethanol accumulation.

Crawford has now (*New Phytol.* **79**, 511; 1977) examined the response of certain seeds to anoxia, for some mire plants can survive for considerable periods as seeds in anaerobic environments. For example the seeds of the rush *Juncus effusus* are reputed to germinate after 7 years submergence.

Sensitivity to prolonged soaking was found to vary with species. Rice seed viability was unaffected by a 6 h soaking, broad bean was reduced to 40% and pea was rendered completely non-viable after such periods.

In the species intolerant of soaking, such as pea and maize, ethanol was always found to be the major product of glycolysis under anaerobic conditions. Malic acid did not accumulate, but lactate was high in the early stages of glycolysis in broad bean and rice, the species tolerant of soaking. Ethanol production, however, exceeded lactate in even these species after 24 h soaking. The overall rate of glycolysis under anaerobic conditions was greater in the sensitive species, hence ethanol accumulated faster in these species.

Thus it would seem that the tolerance of seeds for prolonged immersion in water depends not upon any metabolic 'switch' as is the case for many roots, but upon the capacity to reduce overall metabolic rate. Crawford has further shown that for five out of six Gramineae species which he kept for 6 weeks as imbibed seeds under anaerobic conditions, there was a reduction in viability with increasing temperature. This would again suggest that increased metabolic activity leads to seed death, probably by ethanol accumulation and the associated membrane changes.

Crawford thus maintains that, although the precise metabolic techniques may vary between species and even between organs, the essential problem involved in the toleration of anaerobic conditions is the control and limitation of ethanol accumulation. □

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on input through the second major afferent pathway to the hippocampus from the septum. Just what conditions lead to these plastic modulatory effects on the second input are not yet clear, however. Further research on these preparations should lead in the near future to a better understanding of plasticity in the hippocampus during learning.

The second function of the hippocampus that received considerable attention was its possible role in the detection of changed environmental circumstances, and in the behavioural adjustments that immediately follow the detection of such changes. While many participants agreed that the hippocampus is involved in the detection of change, they differed about the nature of the particular events whose change was detected. For example,

O. S. Vinogradova (USSR Academy of Sciences Biological Centre, Puschino-on-Oka) suggested that the hippocampus is involved in the detection of novelty (that is, any change in stimulus input), J. A. Gray (University of Oxford) that the hippocampus is involved in detecting changes in reinforcement schedule—in particular, from reward to non-reward, and J. O'Keefe (University College, London), on the basis of a theory that he and L. Nadel have proposed, suggested that the hippocampus is involved in setting up spatial maps of the environment and in detecting mismatches between the environment and the map when the former is changed. Given the extent of the agreement that the hippocampus plays a part in the detection of stimulus changes, one hopes that agreement as to the nature of these

changes is not too far off.

A third proposal was the hypothesis that the hippocampus is involved in certain types of spatial information processing. O'Keefe and A. H. Black (McMaster University) presented both single cell and lesion data in support of the O'Keefe and Nadel spatial theory of hippocampal function mentioned above. For example, certain hippocampal cells seem to fire only when a rat is in a particular location in a given environment, and their firing is independent of the rat's behaviour. D. Olton (Johns Hopkins University) also presented lesion data which were consistent with the view that the hippocampus is involved in spatial memory. He trained rats on an apparatus which consisted of a central circular platform from which eight arms radiated. Food pellets were placed

at the end of each arm. Normal rats very quickly learned to retrieve all eight pellets without re-entering an arm in which they had already found food. Rats with damage to the hippocampal formation failed to do this. But their performance, according to data provided by G. Winocur (Trent University, Ontario), improved if the floor of each of the eight arms was identified by a salient cue. It would seem that the lesioned rats could not employ spatial cues provided by the environment outside the maze to identify the location of each arm but could use specific non-spatial cues on the floor of each arm to do so. Although it is still too early to evaluate the spatial hypothesis, it seems to have aroused considerable interest—and controversy.

A final point concerns the problem of species differences. There are marked differences among species in a variety of aspects of hippocampal function, most of which are as yet unexplained. For example, the environmental events which elicited hippocampal single unit activity in the rabbit seem to differ markedly from those in the rat. Also, as C. Vanderwolf (University of Western Ontario) noted, more hippocampal theta electroencephalographic activity unrelated to movement occurs in the rabbit and cat than in the rat and dog. Perhaps the most important species differences are those between man and the other animals. A striking feature of the effects of hippocampal damage in man is an apparent memory deficit; experiments on the other animals show no memory deficit in most situations. Is this a real species difference or is it a procedural artefact? Winocur suggested that it was the latter. According to his view, after hippocampal damage subjects cannot use contextual cues properly to retrieve information in memory. The difference between man and the other animals arises because human subjects are usually tested on a series of learning tasks which interfere with each other unless contextual cues are used to isolate the tasks, while animals are tested on single tasks for which no interference occurs. Winocur argued that if the same type of task had been used in both cases, the deficit would have been similar. L. Weiskrantz (University of Oxford) suggested, however, that the difference may be real. He proposed that the hippocampal changes result in a dissociation between levels of processing. For example in a simple associational task, the human subject cannot talk about what he has learned to associate even though the associational learning is intact. Presumably, animals show no memory deficit because they lack the different levels and communication between levels of processing does not

occur. Which, if either, of these conjectures is correct remains to be seen. In any case, the concern with the attempt to integrate the human and non-human literature is a most important aspect of the problem of species differences and it is gratifying to see so much concern with it at present. □

Sociology of astronomical innovation

from David W. Hughes

SOCIOLOGY is a subject usually omitted from the degree courses of science and engineering students. They are apparently missing a lot; the fascination of the discipline is ably illustrated by a recent paper in the *Quarterly Journal of the Royal Astronomical Society* (18, 326; 1977) by David Edge of the Science Studies Unit, University of Edinburgh.

Edge applies himself to the problems of innovation—the processes of scientific discovery, the invention and introduction of new theories—and he discusses the ways in which astronomy compares and contrasts with other sciences.

Thomas S. Kuhn (*Sociology of Science*, Penguin Books, 1972) has asserted that the general run of scientific activity mainly consists of attempts to force nature into the conceptual boxes supplied by professional education. The scientist is busily accumulating systematically related knowledge within established areas, he is the objective, open-minded searcher after truth, the explorer of nature, the person who rejects prejudice at the threshold of his laboratory. During his education the scientist acquires a set of standards and a collection of mental tools and manual techniques which he can subsequently deploy in his own creative work. He is a puzzle-solver rather like a chess player. Education simply provides him with the rules of the game being played at the moment, initiating him into a pre-established problem-solving tradition without inviting him or equipping him to evaluate that tradition.

Much research is fettered by preconceived convictions and seems to demand a deep commitment to the *status quo*. A belief in the probable success of a venture is obviously an

important motivating factor. Very few scientists would design and build elaborate special-purpose apparatus or would spend months trying to solve a particular differential equation without a quite firm guarantee that their efforts had a high probability of success and would lead to publications and increased status amongst their scientific peer group. The mature scientist can anticipate with considerable precision the sort of result his research should lead to. Knowing what to expect he is then in the ideal position to recognise the anomalous event.

Because gremlins and temporary faults crop up in the large majority of research projects a new discovery often only results when the failure stubbornly refuses to go away. The resolutions of these failures often bring accepted theories and experimental techniques into question thus making the scientific discoverer a controversial figure, a perpetrator of revolutionary thoughts and a swimmer against the normal tides of scientific conservatism.

Very few scientific innovations are made simply by extrapolating from the known. Most are accidental. However, discoveries are not just lying around ready to be tripped over by the aimless wanderer. A high degree of scientific skill is required to discriminate between the key to a new discovery and mere system (or brain) failure.

David Edge has paid special attention to innovations in modern astronomy. His first point is that the large majority of the innovations were not made by astronomers at all but by people who are marginal to the profession. For example, the pioneer British radioastronomers were all physicists, most of them working on applied research. Interestingly the astronomical community at the time tended to ignore the discoveries, and it was many years before a single astronomer changed his experimental strategy and adopted the new radio technique.

The mobility of physicists also seems to be a crucial factor in astronomical innovation. Their experimental competence enabled them to produce results in, for example, the radio, radar, infrared and X-ray fields for an astronomical audience who were not so equipped. Also physicists were not fettered by the stereotype view of the stars and the Universe inculcated into the minds of the professional astronomers. Again innovators seem to thrive if they are one of a large group of scientists (a university department for example) which can provide support for the push into the unknown.

Most astronomical discoveries have been serendipitous, a chance happening in the pursuit of something else. Far too infrequently did discovery come from

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the logical progression of hypotheses, the prediction of a possible new kind of observation, the design, construction and the use of an apparatus to search for the predicted phenomenon and the eventual success, the discovery. In recent times only the discovery of the 21-cm hydrogen line fits this idealistic progression.

A more controversial point in Edge's paper concerns the reception of innovation by scientists. In some disciplines it seems that innovators are treated with apathy and hostility, forced to organise their own societies and journals, mainly because it was thought that the innovation tended to devalue the importance of the hard won skills and competence of established practitioners. Here astronomy seems to be anomalous, most innovators being happily supported and encouraged. Maybe this is because the astronomical innovators are not competing for scarce research resources against members of the established discipline. The pioneers of radioastronomy, for example, were supported by physics and engineering groups.

The final point concerns the strategy of a scientist when faced with a range

of options for future research. To undertake an obvious experiment is to risk outright competition with other workers and possible loss of priority. Attempting a risky or speculative experiment, however, courts failure and also lessens enormously the audience which can appreciate its significance. Most scientists aim for the middle course, trying to prevent duplication and direct competition and trying to ensure success. Innovation, however, opens up a new era into which researchers rush, thus leading to a brief spell of outright competition and its concomitant secrecy.

Edge's paper helps lay to rest the scientists' impression of sociologists as a happy band rambling through science and occasionally stumbling on the obvious with shrieks of amazement. Does an understanding of sociology help the individual scientist? David Edge parries this question by answering that "if you want the (scientific) game to go better, then it is surely necessary to be clear about what kind of game it is". So we may conclude that the sociology of this subject is important, is useful, and should be more widely available to the scientific student. □

kaemic cells were lightly fixed with glutaraldehyde; these were then used to immunise mice, and subsequently to assay antibody binding activity. When the spleens from immune mice were chopped into fragments and placed in microwell cultures, the antibody produced from each fragment could be assayed and from other studies it has been shown that under appropriate conditions each culture well contained one or fewer antibody-producing clones. The second key point of technique was the use of an iodinated rabbit anti-mouse immunoglobulin probe to detect antibody binding to the glutaraldehyde-fixed target cells. Under optimal conditions of spleen fragments culture and microassays for antibody binding, it was possible to obtain sufficient antibody from a single culture well to perform 2,000 assays. In practical terms it means that this technique can provide tumour-specific antibody (by selecting the appropriate culture well) which is tailored to suit the requirements of an individual patient, and in sufficient amounts to monitor accurately the numbers of leukaemic tumour cells during the course of therapy. In short, this technique could provide a major step forward in the management of tumours where chemotherapy is effective but must be closely regulated according to the tumour load.

Serological analysis of human melanoma antigens using autologous sera and cultured melanoma cells from tumour-bearing patients has revealed a variety of antigenic specificities (A. Deleo, Sloan-Kettering). A similar conclusion was reached by S. Ferrone (Scripps Clinic) who tested sera from melanoma patients against a panel of five cultured melanoma cell lines. In both studies there was no evidence of a common tumour-specific antigen as defined by patient sera. However, R. Reisfeld (Scripps Clinic) has shown that a single glycoprotein linked to β_2 -microglobulin can be shown to carry melanoma-specific antigens as defined by a heterologous rabbit antiserum. Putting the available data together, the tentative suggestion emerged that while one, or a few, molecules may carry several antigenic determinants which are tumour specific, there is substantial variation between individuals in the particular constellation of antigens present. In fact one wonders if the HLA system, in which three closely related molecular species carry a cluster of antigenic determinants which vary from one individual to the next, may be an important clue to introducing some order into this field. However, in the absence of genetics, improved chemistry is required to separate these related molecules, and improved serology to elucidate the relationships. □

Analysing tumour antigens

from Rod Langman

The Armand Hammer Cancer Workshop on Tumour Antigens was held at The Salk Institute, San Diego, on 26-29 September, 1977.

A SMALL group representing a wide range of interests met to discuss various aspects of tumour antigens. Classical serological techniques have provided the backbone of current knowledge, but as finer distinctions become necessary the limits of conventional technology in raising and absorbing antisera are being approached. This point was particularly well made during the discussion of chemically-induced tumour antigens. For example, A. Deleo (Sloan-Kettering) used a methyl cholanthrene-induced tumour of Balb/c mice (Meth-A), which had no detectable C-type virus antigen expression, to immunise syngeneic and semi-syngeneic mice. He found that what should be tumour-specific antisera had high levels of nonspecific activity as assayed on a wide range of normal and tumour tissues. These antisera were apparently loaded with antibody against C-type

viral antigens which was produced when, under the stress of immunisation, endogenous C-type virus was induced. Despite extensive absorption the residual tumour-specific antibody still showed unexplained cross reactions that could not be further analysed. H. Festenstein (London Hospital) reported that the methyl cholanthrene-induced P815 tumour has apparent alterations in the H-2 alloantigens, as detected by standard reference antisera. From this and a number of similar observations it was proposed that tumorigenesis may involve modulated expression of H-2 alloantigens. While this interpretation was not widely accepted at the workshop, no competing explanation could be offered. Without better defined monospecific antisera it seems that antigens on chemically-induced tumours will remain elusive.

Although an obvious approach to preparing monospecific antibody, the Milstein-Galfre myeloma hybridisation technique has not yet been applied to tumour antigens. However, R. Levy (Stanford University) did introduce a variant of this idea when he described his adaptation of the Klinman spleen-fragment method for preparing antibody to human leukaemic cells. First, large numbers of the patient's leu-

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Isoscalar breathing mode states identified in ^{144}Sm and ^{208}Pb

from P. E. Hodgson

OVER the past few years many collective multipole excited states of nuclei have been found, mainly by inelastic scattering measurements. The energy spectra of the inelastically-scattered particles show separate peaks at the higher energies due to the excitation of the low-lying states and a broad peak at lower energy due to the excitation of multipole resonances. The multipolarity of the resonance can usually be found by studying the angular distribution of the resonance peak, and comparing it with the distribution calculated from a model of the resonant state with various assumed multipolarities. In this way much has been learnt about the giant quadrupole resonance, but so far it has proved difficult to identify the monopole or breathing-mode state. This is called the breathing mode because it corresponds to the nucleus changing size but not shape, and this shows that its energy is closely related to the nuclear incompressibility.

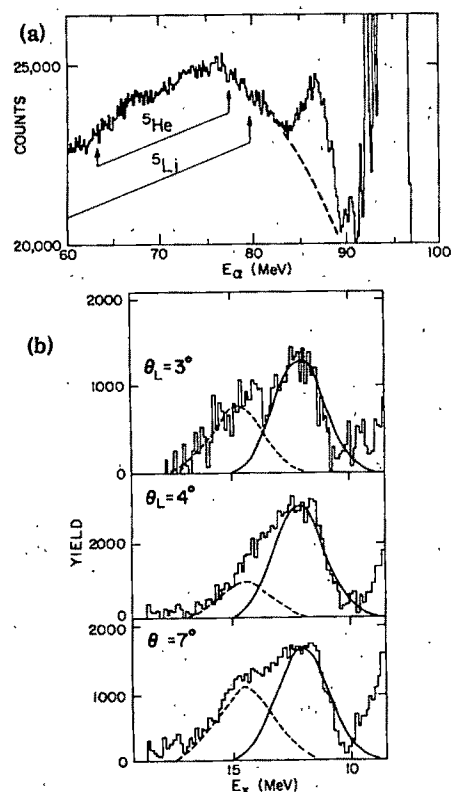


Fig. 1 Energy spectra of the α particles inelastically scattered from ^{208}Pb (a) and ^{144}Sm (b) showing the analysis of the giant resonances into two peaks.

In a recent experiment, Youngblood and colleagues of Texas A&M University have found that inelastic α -particle scattering at forward angles provides clear evidence for the breathing-mode excitation in ^{144}Sm and ^{208}Pb (*Phys. Rev. Lett.* **39**, 1188; 1977). In their earlier work with 97 and 115 MeV α particles they had found that the giant resonance peak has two components, but both had the same angular distribution and were assigned to the giant quadrupole resonance.

The new experiments were made with 98 MeV α particles, and measurements were made at much smaller angles than before, between 3° and 8° to the incident beam. Some of their results are shown in Fig. 1, together with the analysis of the giant resonance peaks into two components. The angular distributions of the two peaks are shown in Fig. 2, and it is clear that they are very different, especially around 4° . The multipolarities of the resonances were found by making calculations of the angular distributions assuming that the states are monopole, isovector dipole, quadrupole and hexadecapole, corresponding to $L = 0, 1, 2$ and 4 respectively. It is clear in each case that the data for the component at the lower energy is well fitted by the quadrupole curve and for the component at the higher energy by the monopole curve in each case. In particular, the predicted signature of the monopole state, a sharp minimum around 4° , is very clear in the data for the excitation to the higher energy for both nuclei, while it is absent in the data for the lower energy excitation.

The giant multipole excitations should satisfy sum rules, which means that the integrated strength of all their components should add to a value that can be calculated from the theory of the excitations. In the present experiments, it is found that all four resonances satisfy the energy-weighted sum rule to within the uncertainties of the analysis. This is a valuable confirmation of the correctness of the assignments.

The results for the breathing-mode excitation energies are in good agreement with previous experimental work, and with the recent theoretical estimates. According to the liquid drop model, the monopole excitation energy is related to the nuclear incompressibility K by

$$E_0 = \pi/3 R (\hbar^2 K/m)^{1/2}$$

where R is the nuclear radius and m the nucleon mass. The excitation energies 13.7 MeV for ^{208}Pb and

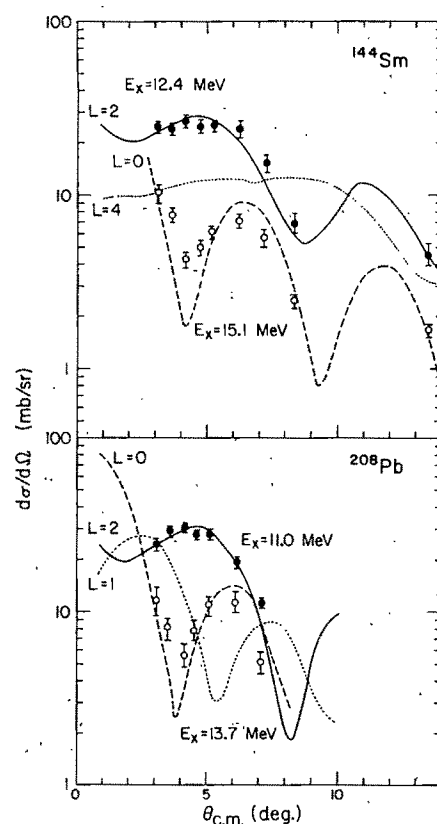


Fig. 2 Angular distributions of the two components of the giant resonance peaks in ^{144}Sm and ^{208}Pb compared with distorted wave calculations assuming various values of the multipolarity L .

15.1 MeV for ^{144}Sm give values of 208 and 197 MeV for K respectively, which are consistent with other theoretical estimates. \square



A hundred years ago

MR. STANLEY will probably arrive in England this week. He has been received with enthusiasm at Rome, Marseilles, and Paris. The Chamber of Commerce and the Geographical Society of Marseilles presented Mr. Stanley with medals. No doubt our own Geographical Society will take the lead in the warm reception which will certainly be accorded in this country to one of the foremost of explorers.

THE wolves in Eastern France have become unusually bold during this winter, and reports are constantly received of their depredations in various parts of the country. In one instance a letter-carrier was driven back by them from his regular route.

From *Nature* 17, 17 January, 232; 1878.

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review article

Why has Regge pole theory survived?

Elliot Leader*

In spite of a very mixed record of success and failure, Regge theory, in which the notion of complex angular momentum is introduced into quantum mechanics still lives on. What has dissuaded physicists from discarding it, and what is our present assessment of the theory?

PHILOSOPHERS of science, who ponder the survival and demise of scientific theories, would do well to test their ideas against the progress of Regge Pole Theory since its inception in 1959.

Tullio Regge's great imaginative leap, the introduction of the notion of a complex angular momentum in non-relativistic quantum mechanics¹, might have ended in oblivion, weighed down by its overpowering mathematical sophistry and rigour, had not S. Mandelstam, seizing upon its crucial element and casting off the enveloping mathematical shroud, demonstrated a direct and striking consequence in the behaviour of high-energy elementary particle collision processes (Mandelstam did not publish this work, but his rôle is acknowledged in many papers).

Thereupon Regge theory really took fire and blazed through the ranks. In no time at all 'Regge pole' (pole in the mathematical sense) had become a household expression. Indeed there is the story of a party at which the charming wife of an American physicist, on being introduced to 'Regge', exclaimed: "Ah Mr Pole! I'm so pleased to meet you at last."

What is it that Regge did? What were the consequences? Where does the matter now stand?

Much of elementary particle physics is concerned with the scattering amplitude F , a function which controls the probable outcome of a collision between two particles. In the simplest case F depends only upon the energy of the collision E and upon the angle θ through which one of the particles is deflected. The behaviour of $F(E, \theta)$ reflects, albeit in a complicated way, the nature of the fundamental dynamical forces acting between the particles; hence its importance.

In ordinary quantum mechanics, where the forces are known, $F(E, \theta)$ is deduced by solving the Schrödinger Equation. Traditionally it was always simplest not to aim for $F(E, \theta)$ itself, but rather to study the collision at a fixed value of the angular momentum l . The forbidding Schrödinger Equation breaks up into a set of much simpler equations, one for each value of l ; l of course, being restricted to positive integer multiples of Planck's constant, as it must be in quantum mechanics. Thereby one obtains a set of amplitudes $f_l(E)$, $l = 0, 1, 2, \dots$, from which it is possible to construct $F(E, \theta)$ itself.

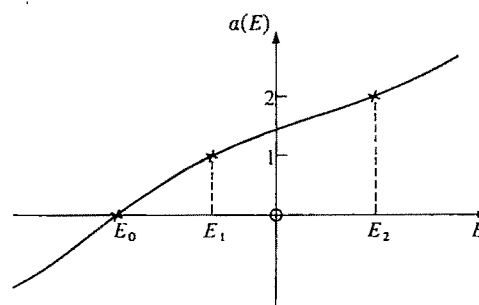
It has long been known that there are certain advantages in adopting the fiction that the energy E can be a complex number, that is, have both real and imaginary parts, and in considering each $f_l(E)$ as a function of the complex variable E (refs 2,3). The remarkable consequence of this bizarre strategy is the following. Suppose that the two particles whose collision is under study are capable of binding together to form a stable bound state, say with angular momentum L and (negative) energy E_B . Then the L th one of the above functions, that is, $f_L(E)$ will be found to have a 'pole' at the negative value $E = E_B$, that is, a simple infinity of the form $1/(E - E_B)$. The continuation of the energy E from its real positive

physical values to complex and negative values has unified the consideration of scattering processes and the existence of bound states.

With hindsight it is obvious that the next, even more bizarre, step ought to have been to try to allow the angular momentum l to take on non-integer, and indeed, complex values. But between the generalisation of the smoothly varying energy E to complex values and a like extension of the variable l , which physically took on only the values $0, 1, 2, \dots$, lay a great psychological barrier. Indeed 33 years were to pass before Regge's brilliant papers laid down the foundation of the theory of complex angular momentum.

Of course, Regge did not complexify for the sake of complexification. It is probable that he saw the introduction of complex l principally as an aid toward proving certain mathematical properties of the scattering amplitude that he had been trying to establish. The harvest was richer than expected.

First there emerged a new way of classifying stable bound states and resonances (quasi-stable states) into families. Instead of focussing separately on a bound state with a given angular momentum, say $l = 0$ with energy E_0 , and then upon one with angular momentum $l = 1$ with energy E_1 , and so on, one now focussed on a single object, the 'trajectory function' $\alpha(E)$ with the property that if a particular value of E should cause $\alpha(E)$ to equal a positive integer, say L , then a bound state would exist at that energy E and with angular momentum $l = L$. Thus in the above example the bound states would correspond to finding $\alpha(E_0) = 0$, $\alpha(E_1) = 1$, and so on. The curve $\alpha(E)$ against E signals the existence of bound states or resonances if and when it passes through integer values.



The bound states at energies E_0 , E_1 , and the resonance at E_2 (positive energy) are united into a new kind of family characterised by their trajectory function.

Second, it was found that just as $f_L(E)$ has a pole, that is, is infinite at the energy E for which a bound state of angular momentum $l = L$ exists, so now $f(l, E)$ has a pole, a 'Regge pole' whenever E is such that $\alpha(E)$ equals the value of l under consideration. The Regge pole coincides with the physical bound

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states or resonances when this value happens to be an integer.

Third, it was established that in the quite unphysical region of very large $\cos \theta$ (that is, $\cos \theta \gg 1$) the scattering amplitude is proportional to $(\cos \theta)^{\alpha(E)}$. In some mysterious way the Regge trajectory function $\alpha(E)$, so intimately linked to the properties of the bound states, is also responsible for controlling the growth of the scattering amplitude in the weird region of large $\cos \theta$.

This, in the broadest of terms, was the achievement of Regge. Whatever he proved is unquestionably true and will remain so as long as quantum mechanics holds sway. Indeed Regge theory has been included in good undergraduate text books for more than a decade.

Beautiful though this all is, it is very heavy going mathematically and might have remained undigested for a long time had not Mandelstam emphasised that in the real world of relativistic physics the quite meaningless limit of large $\cos \theta$ in the reaction $A + B \rightarrow A + B$ is closely related to the physically sensible, and exceedingly interesting, limit of high energy in the related reaction $A + \bar{A} \rightarrow B + \bar{B}$ involving the antiparticles \bar{A} , \bar{B} of A and B .

The full story is technical and difficult, but what emerges is remarkably simple and quite dramatic: the behaviour of the scattering process $A + B \rightarrow A + B$ at very high energies E is proportional to the energy raised to the power $\alpha(t)$ that is, $E^{\alpha(t)}$, where α is the Regge trajectory connected with the bound states and resonances of the $A\bar{A}$ or $B\bar{B}$ systems. (t is the square of the 'momentum transfer' in the scattering—roughly $(E\theta)^2$).

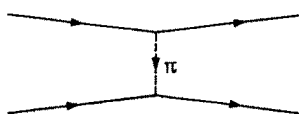
Two points are noteworthy: that there is such a close link between high energy behaviour and the properties of the bound states and resonances, and that the behaviour thus predicted at high energy, in which the power to which E is raised depends upon t (and therefore upon θ), is quite in contrast with what had been expected.

Like theologians of old taunted by the threat of a heliocentric solar system, the experimentalist rushed forth to destroy the theory. Amazingly, the first results from CERN in 1961 seemed to support the theory. Proton-proton scattering was changing with energy as predicted. (The diffraction peak was shrinking.) Enraged, the experimentalists tried harder. (There was the famous slide, shown at a conference in 1963, in which a certain experimentalist appears imperiously surveying his apparatus with one raised foot thrust firmly on top of a large dark rectangular box—said to be the coffin of Regge theory.) Of no avail; the physical world seemed to be in tune with Regge, and remained so for some time.

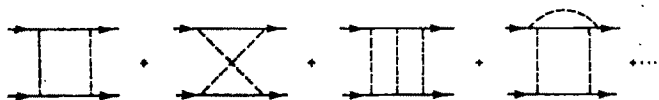
Then the reaction set in. The experimentalists, exploring all the different reactions they could think of, discovered behaviour that was sometimes not quite what was expected. The theorists, probing the intricacies of the argument, found all sorts of problems in going from the non-relativistic to the relativistic situation. Hundreds of papers were written, spawning a rich and exotic vocabulary. Trajectories could bear 'daughters'; trajectories might 'conspire' together; 'ghost-killing' mechanisms would have to operate at certain 'nonsense' points. And so on.

Many of these points have never been resolved fully, but what emerges as the main difficulty in Regge theory can be understood heuristically as follows.

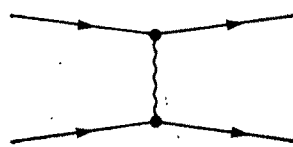
Quantum field theory taught us, long ago, to regard scattering processes as 'mediated' by the exchange of 'virtual' particle, with some well-defined spin, a π -meson say, with zero spin,



and bequeathed to us the impossible task of summing up the effects of exchanging these virtual particles in all possible ways.

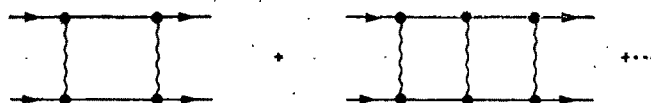


The expression given by Regge Theory, drawn symbolically as



miraculously represents, at high energies, the sum of a huge subset of these diagrams, and can be thought of as representing the exchange of a composite object a 'Reggeon' with variable spin—the rôle of the spin being played by the ubiquitous $\alpha(t)$.

But though infinitely large, this subset does not, alas, exhaust all the diagrams, and we are forced to grapple with multi-Reggeon exchange diagrams such as



The theory becomes difficult and subtle—a new form of field theory—but at least one relieved of some of the worst idiosyncrasies of ordinary field theory. For example, in ordinary field theory it is impossible to allow the exchange of a particle whose spin is greater than one unit, because doing so triggers the appearance of unmanageable infinities in the calculations. But Reggeons with arbitrarily high spin can be exchanged with equanimity.

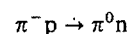
Reliable results for diagrams with multi-Reggeon exchange are hard to come by. It does seem, though, that adding in the results of multi-Reggeon exchange gives answers substantially different from the single Regge pole results. If so, what on earth does the good agreement between some experiments and single Regge pole exchange mean?

That is the present conundrum. Why do some experiments support Regge poles? Why do others disagree? And is the disagreement in a direction that could be explained by multi-Reggeon exchange?

In the past few years there have been two shifts of emphasis in the experimental work.

(1) Improved techniques of detection and the availability of more intense beams of elementary particles from the new accelerators have made it possible to study more refined aspects of collision processes. Rather than simply asking with what frequency particle C emerges at angle θ in the reaction $A + B \rightarrow C + D$, one can now ask also about its 'polarisation' (the direction of its spin), or even about the correlation between the direction of its spin and that of D .

Regge pole theory makes very precise predictions for these measurements—and they are generally wrong. A marvellous example is the reaction



in which the colliding positively charged proton and negatively charged π -meson effectively swap electric charges, emerging as an electrically neutral π -meson and neutron. It can be demonstrated quite rigorously that one, and only one, kind of Reggeon can be exchanged in this process. Thus the usual complication arising from the adding of the effects of several Reggeons is absent and the predictions of pure Regge pole theory are laid bare, open, one might have believed, to the ultimate judgement.

The measured angular distribution of the emerging particles and the manner in which this changes with energy provide a brilliant success for the theory. Even the shape of the angular distribution (see Fig. 1) with its changing sharp minimum is understood as a consequence of the structure of the theory. (The trajectory involved is that of the ρ -meson family and the point at which the minimum occurs corresponds to the value of t at which

$\alpha_p(t) = 0$). But the spin of the neutron, predicted quite firmly to be orientated randomly, with no specially preferred directions, has now been found to point predominantly in one particular direction (it is polarised) thus dealing a grievous blow to the theory.

Another example of great interest is the pair of reactions

$$\pi^+ p \rightarrow \pi^+ p$$

$$\pi^- p \rightarrow \pi^- p$$

involving the collision of π -mesons of positive or negative electric charge with protons. For reasonably small angles of scatter one expects little difference between the two reactions, and this is confirmed experimentally, but for large angles of scatter (close to 'backward' scattering) Regge theory predicts significantly disparate behaviour. The reason is simple. In the reaction $\pi^- p \rightarrow \pi^- p$, at large angles of scatter, the only Regge pole of importance is that belonging to the family of nucleon resonances usually labelled Δ (the first member is the spin 3/2, isotopic-spin 3/2 resonance with mass 1,236 MeV), so the dependence upon energy in backward $\pi^- p \rightarrow \pi^- p$ is proportional to E raised to the power α_Δ . On the contrary, in the reaction $\pi^+ p \rightarrow \pi^+ p$ at backward angles, two Regge poles play a part, one belonging to the Δ family and the other to the family of the nucleon itself (labelled N), and their effects must be added. Are they equally important? The shape of the angular distribution in $\pi^+ p \rightarrow \pi^+ p$ (see Fig. 2), with its very sharp minimum close to point of completely backward scattering ($\theta = 180^\circ$), would emerge naturally from the theory if the N Regge pole dominated. (The dip would just correspond to the point at which $\alpha_N(t) = 0$). Our conclusion: at medium energies, where the processes have so far been measured, the N Regge pole dominates, and since it only

Fig. 1 The angular distribution in $\pi^- p \rightarrow \pi^0 n$ at several energies⁴, ∇ , CERN, 1968 5 GeV; Δ , CERN, 1965 10 GeV; \blacksquare , HEP, 1974 21 GeV; \circ , Fermilab, 1974 40.6 GeV; \bullet , Fermilab, 1974 101 GeV; \square , Fermilab, 1974 200 GeV.

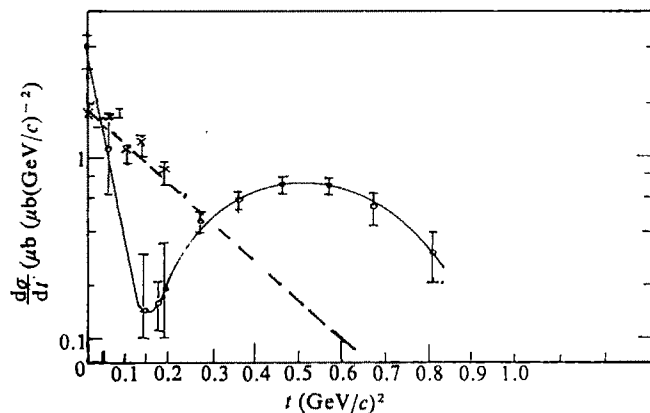
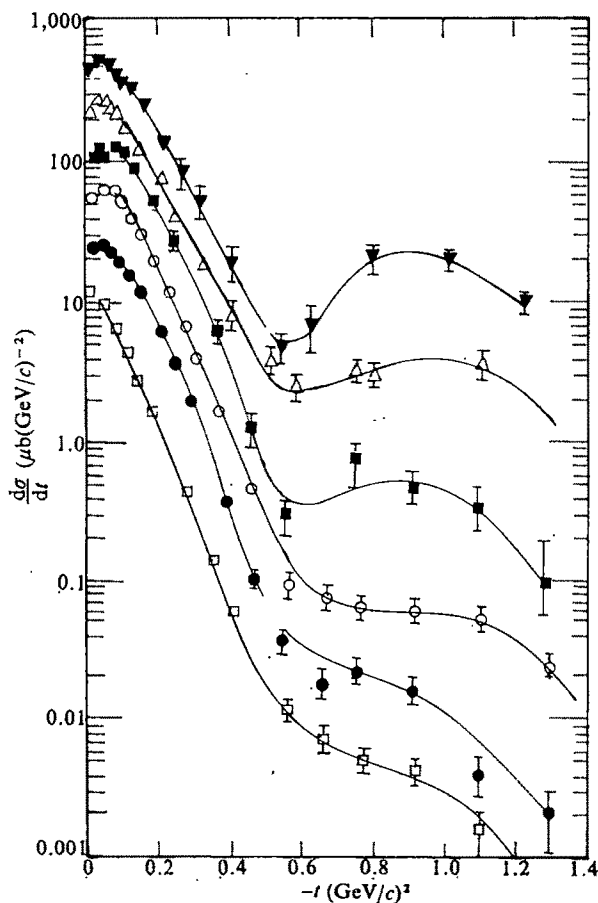


Fig. 2 The angular distributions for $\pi^- p \rightarrow \pi^- p$ (---) and $\pi^+ p \rightarrow \pi^+ p$ (—) near the backward direction (eye fits)⁵.

contributes to one of the reactions, that is, to $\pi^+ p \rightarrow \pi^+ p$, we have a natural explanation for the striking difference between the angular distributions of the two reactions (Fig. 2).

But now comes the rub. From the properties of the two families of particles, N and Δ , we know that α_Δ must be numerically greater than α_N . Thus although at present energies the N contribution dominates in $\pi^+ p \rightarrow \pi^+ p$ this cannot continue to be true at higher energies. For the Δ contribution is growing like E^{α_Δ} , compared with E^{α_N} for the N, and is bound to overhaul the N and eventually dominate at very high energies.

We thus have a dramatic prediction for very high energies. Both reactions $\pi^+ p \rightarrow \pi^+ p$ and $\pi^- p \rightarrow \pi^- p$ will be dominantly controlled by the same Regge pole Δ . Their angular distributions, so dissimilar at medium energies, will then look alike, and will take on the shape at present shown by $\pi^- p \rightarrow \pi^- p$.

A splendid and challenging prediction! But preliminary results at fairly high energies (about 70 GeV) show no change at all. The $\pi^+ p$ angular distribution is just as sharp and spiky as it ever was. Very careful measurements are planned at Fermilab at energies of 100–200 GeV. Their outcome is eagerly awaited.

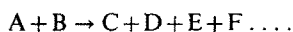
As a last and most mysterious example consider neutron-proton collisions in which the neutron bounces back at very large angles. Although the experiments are difficult (because it is hard to know exactly how many neutrons are arriving per second in one's beam) it does seem to be established that the energy dependence in this reaction is most peculiar. It starts off at low and medium energies behaving as if it was controlled by the trajectory α_π belonging to the Regge family of the π -meson. As α_π is approximately equal to zero, E^{α_π} gives hardly any change at all as the energy increases. But suddenly, at energies in the region of 60 GeV, it begins to grow. Now some growth with energy is not at all surprising. In fact, it is predicted, because the trajectory α_ρ of the ρ -meson Regge family, should also play a part, and its numerical value is about $\frac{1}{2}$, yielding a contribution increasing like $E^{1/2}$. But the growth discovered experimentally seems to be far more rapid; some would even claim it proportional to E itself.

If this were to be substantiated it would really upset the apple-cart—for the number one, to which power it is claimed E is raised, is in Regge theory somewhat sacrosanct. It is the attribute only of that mysterious trajectory, the 'Pomeron', which so far as we know, is not connected with any family of particles or resonances, and which possesses the virgin properties of the vacuum itself—no electric charge, no baryon number, no isotopic spin, no strangeness.

But the Pomeron's rôle is limited to 'diffractive' processes; elastic reactions like $pp \rightarrow pp$ or $np \rightarrow np$ in which the projectile is scattered through a very tiny angle. In our example of large angle or backward scattering it is impossible to exchange a Pomeron. Whatever we exchange must be electrically charged and have a non-zero value of isotopic spin.

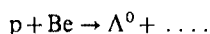
What should we conclude? Are the experiments wrong? Or if they are correct, are we discovering the existence of pretenders to the Pomeron throne? Is there perhaps a family of privileged Regge poles, with trajectories like the Pomeron's, but with different sets of charges, isotopic-spins and so on? (We have, in a moment of fantasy, already speculated upon the existence of such objects—and called them 'Odderons'⁶). Or, finally, is the unexpected energy dependence simply a signal of the failure of Regge pole theory?

(2) The existence of very high energy beams of particles has stimulated interest in reactions in which many particles are produced in the collision:



It is not generally possible to identify and analyse the properties of all the particles emerging, so, often, attention is focussed upon just one of them. (Measurements of this type are rather inappropriately referred to as 'inclusive' experiments.) A very clever argument of A. Mueller⁷ shows that many of the properties of inclusive experiments ought to be controlled by the same Regge poles that are active in the simpler reactions we were considering earlier. Many measurements, principally of the angle and momentum of one of the emerging particles in inclusive experiments, have agreed nicely with the predictions, and have yielded Regge trajectories in good agreement with those determined from simple scattering processes. But recently, a new phase in the experiments, in which the spin of the particle is also measured, has begun to provide some surprising results in contradiction with Regge pole theory.

The best example is probably one in which protons collide with a Beryllium target at 300 GeV to produce neutral lambda particles (Λ^0) plus much debris that is ignored:



Normally it is exceedingly difficult to determine the direction of a fast particle's spin, but it is a peculiar favour of Nature that when the Λ^0 , which is unstable, decays into a proton and a π^-

meson, one can determine the spin-direction which the Λ^0 had from an elementary study of the angles of emergence of these decay products. (The Λ^0 is said to be 'self-analysing'.) According to Regge pole theory the Λ^0 should not be polarised. Experimentally, however, there is a definite favouring of one particular direction for the spin.

Where, finally, do we stand?

As regards Regge's own contribution, the introduction of the concept of complex angular momentum into quantum mechanics, that, as we have said, is inviolate. But the generalisations to the relativistic domain of elementary particle physics are fraught with difficulty and have a mixed record of success and failure. Many indeed are the predictions of angular distributions and their change with energy that have been confirmed experimentally, but many too are the cases of disagreement.

Given this state of affairs, is it conceivable that Regge pole theory is correct? In its simplest, naive form, with the exchange of single Reggeons, there is no hope. In its broader guise, with multi-Reggeon exchange, we simply do not know the answer. Sometimes it looks as if results are in the right direction but the calculations are too difficult, and any confrontation between experiment and these calculations could just as well reflect inaccuracy in our approximations as some basic fault of the theory.

Strangely though, despite these uncertainties, and even if the theory may be wrong, the language of the theory is likely to survive for a long time to come. For, stripped of all its details, Regge pole theory does focus upon a link between the behaviour of scattering and the quantum numbers of the 'object' that can be exchanged. And this, at a qualitative level, does seem to be a correct and useful concept. But the elucidation of the detailed dynamical properties of this 'object' will have to await a new Regge.

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articles

Reflection of X rays by neutron star surfaces

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A neutron-star surface should act as a good reflector for photons up to hard X-ray energies, due to the high density of the magnetic surface matter. Reflectivities are high, especially in spectral bands above the plasma and cyclotron frequencies depending on the photon polarisation. X-ray reflection may be important for the beaming of the binary neutron star Her X-1 and similar objects.

X RAYS are reflected under glancing angles by flat surfaces of condensed matter. This phenomenon is based on the fact that, for X-ray energies away from absorption edges the electrons can be considered as free particles such that the photons interact with them coherently. At frequencies ω larger than the plasma fre-

quency $\omega_p = (4\pi e^2 n_e / m_e)^{1/2}$, the refractive index $n = \{1 - \omega_p^2 / \omega^2\}^{1/2}$ is real and smaller than unity. Consequently, total external reflection occurs at glancing angles $\alpha < \alpha_c = \cos^{-1} n$. For terrestrial materials $n_e \sim 10^{24} \text{ cm}^{-3}$ and α_c is $\sim 2^\circ$ for $\sim 1 \text{ keV}$ photons.

According to current concepts (see, for example, ref. 1) a neutron star surface consists of a solid or liquid magnetic polymer. With respect to its reflective properties two radical changes occur in comparison with terrestrial matter. First, the surface electron density is $\propto B^{6/5}$ and will be of the order $\sim 10^{28} \text{ cm}^{-3}$ for $B \sim 10^{12} \text{ G}$ fields². This leads to a plasma frequency which is ~ 100 times larger than for normal matter. Consequently, glancing reflection should occur up to $\sim 100 \text{ keV}$ at 2° , or up to much larger angles at lower energies.

Second, the electron plasma is highly anisotropic since the

electron can move freely only along the magnetic field lines while their motion is quantised in the transverse direction. Consequently, the refractive and reflective properties of the surface will strongly depend on the magnetic field orientation and on the electron cyclotron frequency $\omega_B = eB/m_e c$.

Both effects depend crucially on the surface magnetic field strength, which is now known³ with some precision for the neutron star Her X-1 ($\sim 5 \times 10^{12}$ G). This fact and the possible effects of X-ray reflection on the beaming of this or other X-ray sources led us to investigate this problem quantitatively. Unfortunately, nothing is known about the macroscopic surface structure of neutron stars. But, it seems reasonable to assume that it is smooth enough to exhibit at least some mirror qualities.

We calculate here the X-ray reflectivity of a neutron star surface as function of photon energy, polarisation, and angle of incidence under idealised conditions. We also discuss possible deviations expected to occur in the case of real neutron star surfaces and summarise some expected observational effects which may have a role for systems like Her X-1.

X-ray reflection at a neutron star surface

We assume a plane surface which represents a sharp transition from vacuum to the high density surface matter. The magnetic field is orientated perpendicular to the surface. The latter will be a good approximation in the vicinity of the accretion column at a neutron-star magnetic pole. As already discussed, the X-ray optical properties of the surface will depend on the electron plasma frequency

$$\hbar\omega_p = (4\pi e^2 n_e / m_e)^{1/2} \hbar \quad (1)$$

and on the electron cyclotron frequency

$$\hbar\omega_B = eB/m_e c \hbar = 11.6 \times B_{12} \text{ keV} \quad (2)$$

The total number density of electrons can be calculated from the nuclei density of the condensed magnetic matter², which results in

$$n_e = 1.24 \times 10^{27} Z_{26}^{2/5} B_{12}^{6/5} \text{ cm}^{-3} \quad (3)$$

Assuming that the surface consists of iron we get for the plasma frequency

$$\hbar\omega_p = 1.45 \times B_{12}^{3/5} \text{ (keV)} \quad (4)$$

We neglect here the atomic binding energies of the electrons which is justified by the fact that for $B \sim 5 \times 10^{12}$ G and iron nuclei most electrons have binding energies smaller ~ 2 keV (W. Hillebrandt, personal communication).

In order to calculate reflectivities we follow the classical approach and use the boundary conditions which must be satisfied on the surface by the electromagnetic fields (see ref. 4). Besides the incident and reflected waves we have to consider two

refracted waves as two modes of propagation, the ordinary and the extraordinary one, exist in the magnetised plasma.

Figure 1 depicts the coordinate system and the notation used. The magnetic field is orientated perpendicular to the surface which is given by the plane $z = 0$, $y = 0$ is the plane of incidence. We neglect the effects of vacuum birefringence in a strong magnetic field^{5,6} and assume an index of refraction $n_0 = 1$ above the surface. This is justified because for a 10^{12} G field the deviations from unity are of the order 10^{-5} . Below the surface the dielectric properties are described by a tensor ϵ . The different waves are characterised by their electric vectors E and wave vector k .

We first consider the properties of the two refracted waves. The refraction indices for the ordinary and extraordinary mode of propagation are given by the dispersion relation (refs 7 and 8):

$$\begin{bmatrix} S - n_m^2 \cos^2 r_m & -iD & -n_m^2 \sin r_m \cos r_m \\ iD & S - n_m^2 & 0 \\ -n_m^2 \sin r_m \cos r_m & 0 & P - n_m^2 \sin^2 r_m \end{bmatrix} \begin{bmatrix} E_{xm} \\ E_{ym} \\ E_{zm} \end{bmatrix} = 0 \quad (5)$$

$$\text{with } R = 1 - \frac{\omega_p^2}{\omega^2} \frac{\omega}{\omega - \omega_B + i\omega_D}$$

$$L = 1 - \frac{\omega_p^2}{\omega^2} \frac{\omega}{\omega + \omega_B + i\omega_D}$$

$$P = 1 - \frac{\omega_p^2}{\omega^2} \frac{\omega}{\omega + i\omega_D}$$

$$S = \frac{R+L}{2}$$

$$D = \frac{R-L}{2}$$

where ω_p , ω_B , and ω_D are the plasma frequency, the cyclotron frequency and the damping frequency, respectively, and the index $m = 1, 2$ describes the ordinary and extraordinary mode. From this dispersion relation we derive the following amplitude ratios:

$$\frac{E'_{mx}}{E'_{mz}} = \frac{P - n_m^2 \sin^2 r_m}{n_m^2 \sin r_m \cos r_m} = \alpha_m \quad (6)$$

$$\frac{E'_{my}}{E'_{mz}} = \frac{-iD \alpha_m}{S - n_m^2} = \beta_m \quad (7)$$

and, as the determinant must vanish, the quartic equation for the two refractive indices:

$$An_m^4 - Bn_m^2 + C = 0 \quad (8)$$

with $A = S \sin^2 r_m + P \cos^2 r_m$

$$B = RL \sin^2 r_m + PS \cdot (1 + \cos^2 r_m)$$

$$C = PRL$$

For propagation modes, both the ordinary and the extraordinary ones, refraction is described by Snell's law

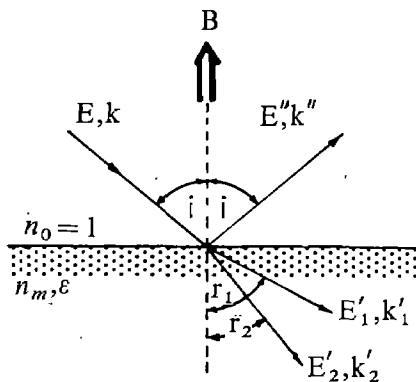
$$n_m = \sin i / \sin r_m \quad (9)$$

in which n_m implicitly depends on r_m .

The angle of refraction r_m can be calculated if we replace n_m in (8) by (9) and solve for $\sin r_m$. Inserting these values into (6) and (7) we find the amplitude ratios α_m and β_m of the electric field components below the surface.

In order to determine the electric field components of the reflected wave we use the boundary conditions at the surface which read in the present case:

Fig. 1 The propagation vectors for refraction and reflection at the plane surface.



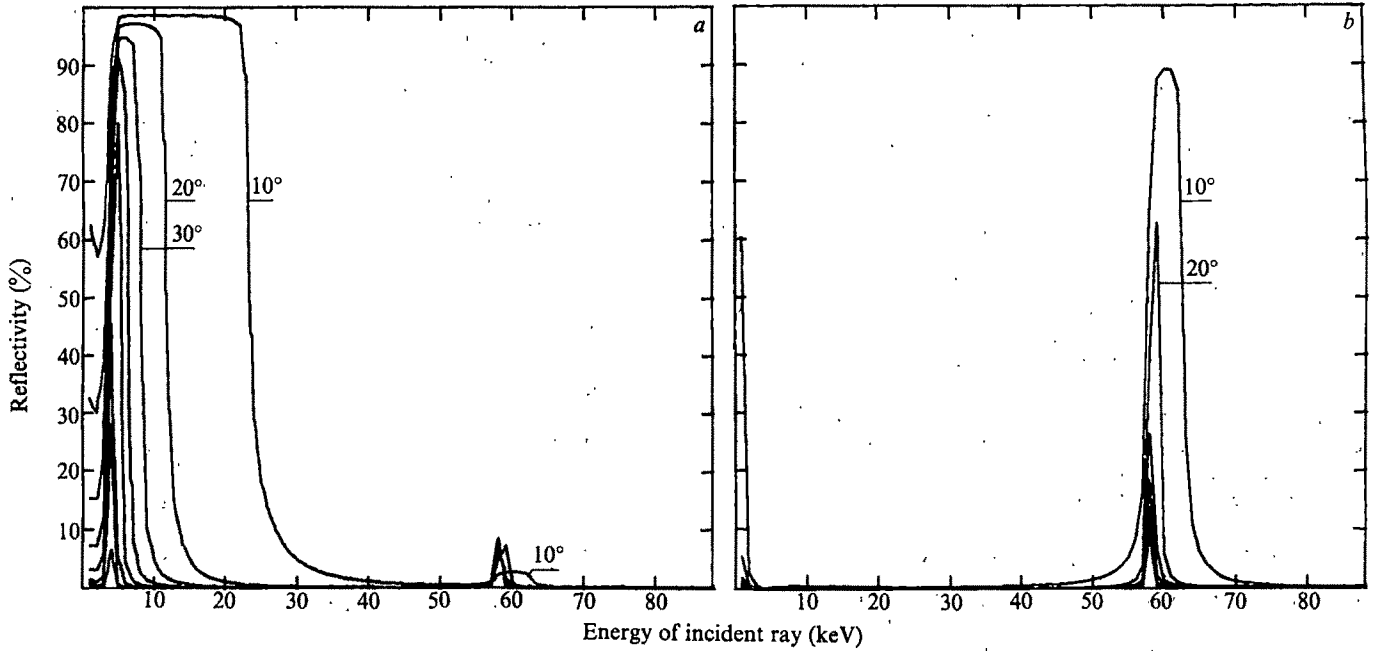


Fig. 2 X-ray reflectivities as function of photon energy for different polarisations of the incoming photon and grazing angles between 10° and 80°. Plasma frequency, 4 keV; cyclotron frequency, 58 keV; damping frequency, 0.1 keV. *a*, Polarisation parallel to plane of incidence; *b*, polarisation perpendicular to plane of incidence.

$$\left[\mathbf{E} + \mathbf{E}'' - \sum_{m=1}^2 \mathbf{E}'_m \right] \cdot \mathbf{Z}_0 = 0 \quad (10)$$

$$\left[\mathbf{k} \times \mathbf{E} + \mathbf{k}'' \times \mathbf{E}'' - \sum_{m=1}^2 \mathbf{k}_m' \times \mathbf{E}_m' \right] \cdot \mathbf{Z}_0 = 0 \quad (11)$$

$$\left[\mathbf{E} + \mathbf{E}'' - \sum_{m=1}^2 \mathbf{E}'_m \right] \times \mathbf{Z}_0 = 0 \quad (12)$$

$$\left[\mathbf{k} \times \mathbf{E} + \mathbf{k}'' \times \mathbf{E}'' - \sum_{m=1}^2 \mathbf{k}_m' \times \mathbf{E}_m' \right] \times \mathbf{Z}_0 = 0 \quad (13)$$

where \mathbf{Z}_0 is the unit vector in z -direction.

Representing \mathbf{E} and \mathbf{E}'' by their components perpendicular and parallel to the plane of incidence, one gets the linear equations:

$$\begin{bmatrix} E_{\perp} \\ E_{\parallel} \\ E_{\parallel} \\ E_{\parallel} \end{bmatrix} = \begin{bmatrix} \beta_1 & \beta_2 & -1 & 0 \\ \frac{n_1 \cos r_1 \beta_1}{\cos i} & \frac{n_2 \cos r_2 \beta_2}{\cos i} & 1 & 0 \\ \frac{\alpha_1}{\cos i} & \frac{\alpha_2}{\cos i} & 0 & 1 \\ \frac{P}{\sin i} & \frac{P}{\sin i} & 0 & -1 \end{bmatrix} \cdot \begin{bmatrix} E'_{1\perp} \\ E'_{2\perp} \\ E'_{1\parallel} \\ E'_{2\parallel} \end{bmatrix} \quad (14)$$

Inverting this system of linear equations gives the dependence of E_{\parallel} on E . The corresponding reflectivities $|E''|^2/|E|^2$ and $|E'_{\parallel}|^2/|E_{\parallel}|^2$ as a function of photon energy are shown in Figs 2 and 3, where we have replaced the angle of incidence i by the grazing angle $\pi/2 - i$.

For the cyclotron frequency the value measured for Her X-1 (58 keV, ref. 3) is used. The plasma frequency was calculated for

the corresponding magnetic field strength (5.3×10^{12} G) using equation (4), which leads to $\hbar\omega_p \sim 4$ keV. The damping frequency in intense magnetic fields has been treated in ref. 9. It turns out that in the present case $\hbar\omega_D$ is of the order 10 to 100 eV, that is small compared to X-ray photon energies. To improve the legibility of the figures in the vicinity of the resonances we arbitrarily used for our calculations a value $\hbar\omega_D = 100$ eV.

Figures 2 and 3 show that for photons polarised in the plane of incidence the reflectivity is near unity in a spectral band between $\hbar\omega_p$ and a cut-off energy depending on the grazing angle. Total external reflection ($n < 1$) takes place in this region. At the cyclotron frequency a small peak occurs which corresponds to the excitation of the extraordinary mode in the surface.

For photons polarised perpendicular to the plane of incidence one finds substantial reflectivity below the plasma frequency and in a spectral band between $\hbar\omega_B$ and a cut-off energy depending again on the grazing angle. Also in this upper energy band the high reflectivity is due to total external reflection ($n < 1$). We note that both spectral bands in which total external reflection occurs are limited by very steep slopes on both sides. If one plots the reflectivity as function of the grazing angle, as shown in Fig. 4, one finds a steep cut-off behaviour here as well.

Application to Her X-1

According to the generally accepted model for Her X-1, the pulsed X rays originate from hot spots at the magnetic poles on the stellar surface which are heated by the accreted material falling down along the polar field lines. It is clear that a beaming mechanism is required in order to account for the sharpness of the observed 1.24-s X-ray pulses, however, there is no agreement regarding the beaming geometry. The basic possibilities discussed are pencil beams (along the polar field lines) or fan beams (perpendicular to the field lines). We regard the fan beam geometry as the more likely possibility since the radiation most easily escapes sideways from the base of the accretion funnel. In this case part of the radiation will hit the stellar surface where it undergoes reflection, as discussed above. The reflected component should lead to sharp structures in the spectral time variability of the source due to the sharp on-sets and cut-offs in reflectivity as functions of grazing angle and photon energy.

Observations show that the 1.24-s pulsations of Her X-1 are characterised by: a double peak structure at low energies (~ 2 to ~ 20 keV, refs 10, 11); single peaks between ~ 20 keV and

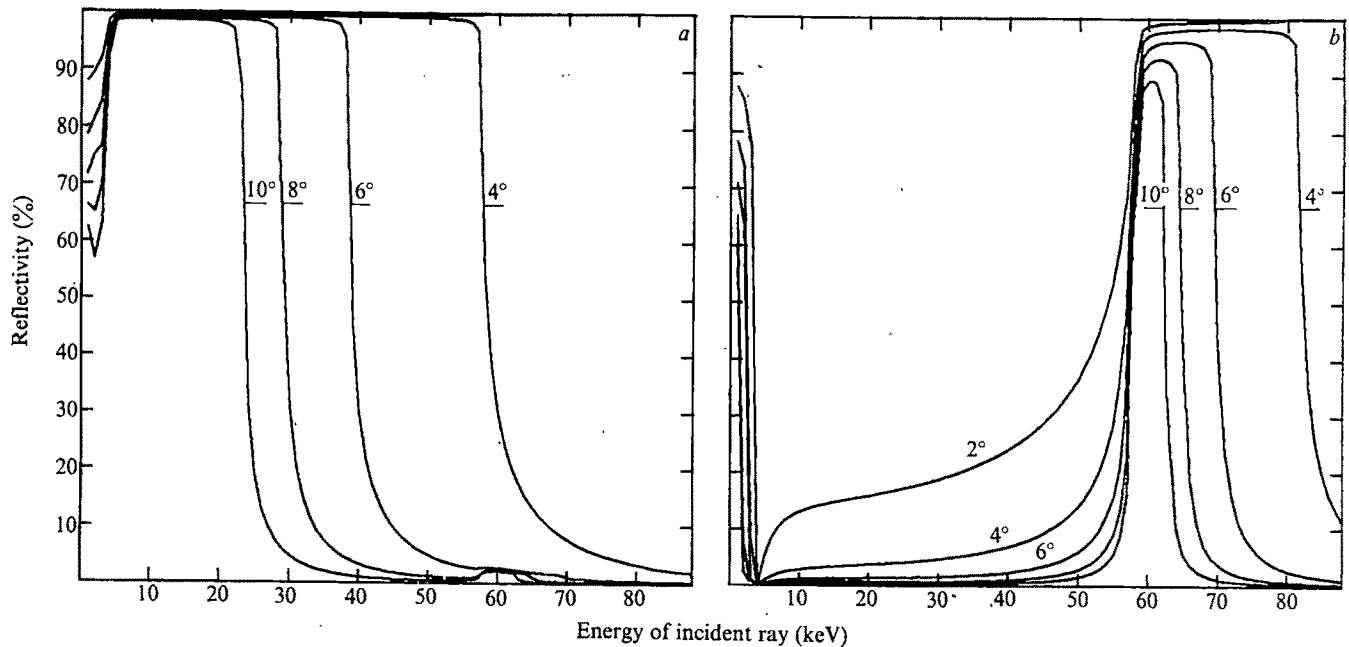


Fig. 3 Same as Fig. 2; grazing angles 4° to 10° .

~ 60 keV, including the cyclotron resonance at ~ 58 keV (ref. 12); and a double peak structure between ~ 60 keV and 90 keV, that is above the cyclotron resonance (ref. 12).

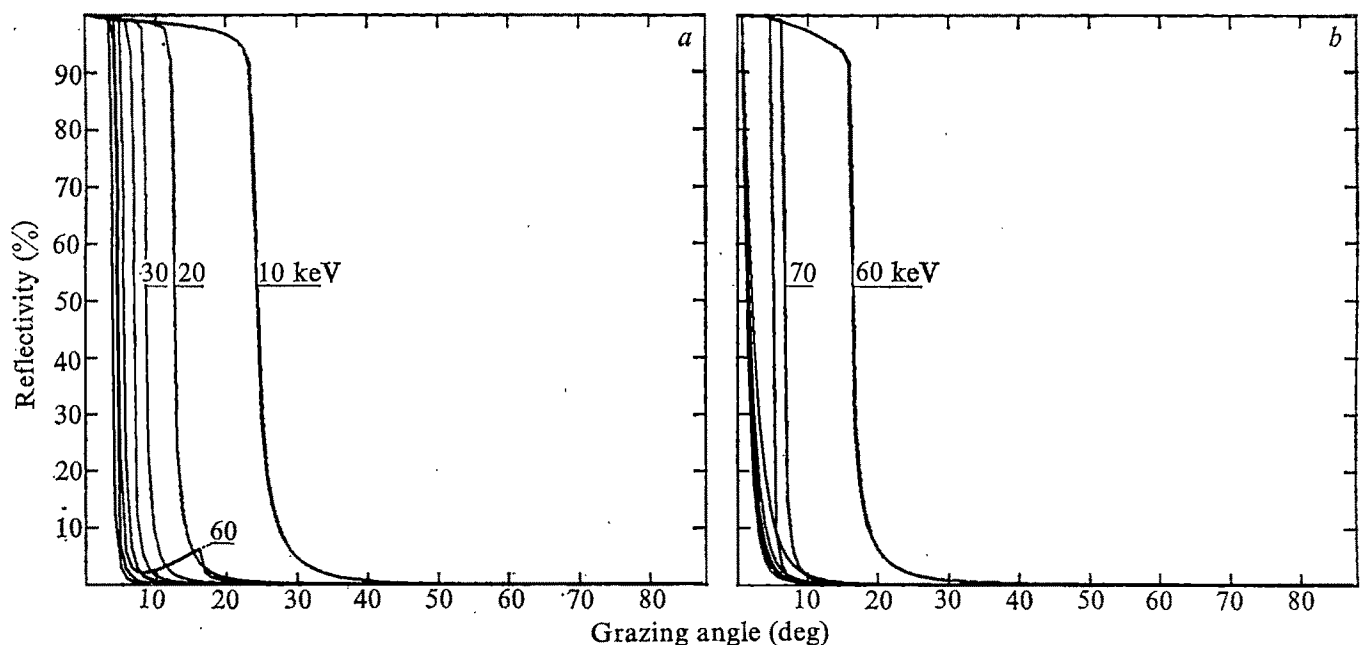
This complex behaviour can be explained by the following simple model. Between 20 and 60 keV one sees the direct, unmodified fan beam which is emitted from the base of the accretion column under a polar angle $\sim \pi/2$. This requires that near pulse maximum the line of sight is approximately at right angles to the polar field lines. In the 2 – 20 keV range and above the cyclotron resonance at 58 keV, an additional reflected beam occurs which has its intensity maximum at $\theta_m < \pi/2$. As the line of sight crosses the reflected beam twice in one rotational period, namely just before and after reaching the fan beam maximum, double pulses are produced which are superimposed on single pulses.

According to Figs 2 and 3 the spectrum of the reflected beam will strongly depend on the polarisation of the incident beam. This polarisation, however, is expected to be zero, as any

polarisation of the X-ray beam emerging from the accretion column will be completely destroyed as the beam propagates through the strong magnetic field near the neutron star due to vacuum birefringence⁶. Therefore, the shape and spectrum of the reflected beam will depend only on the shape and spectrum of the fan beam, and not on its polarisation properties at emission which may be very complex.

Our considerations will not be altered substantially if we drop the assumption of a plane mirror with perpendicular magnetic field made above and consider the spherical polar cap of a neutron star with a dipolar magnetic field. A considerable deterioration of specular reflection, however, will be caused if an atmosphere exists which is dense enough to scatter the photons incoherently before they reach the high density surface. The answer to the question of whether such an atmosphere exists will depend on the cohesive energy of surface matter and on the surface temperatures. At 5×10^{12} G the cohesive energy of the linear chains which form the condensed matter is ~ 8 keV (ref. 2). In the immediate

Fig. 4 X-ray reflectivities as function of grazing angle for different energies and polarisations of the incoming photons. *a*, Polarisation parallel to plane of incidence; *b*, polarisation perpendicular to plane of incidence.



surroundings of the radiating accretion column the surface temperature may be above this value due to heating by the refracted photons and Thomson scattering recoils. Also heat conduction in the stellar material may play a role in this context. One, therefore, expects that up to a certain radial distance from the accretion column the neutron star surface is covered by a dense atmosphere and represents a 'tarnished mirror', reflecting quasi-isotropically by Thomson scattering. Specular reflection will occur in a ring zone between this region and the neutron star horizon as seen from the radiation source.

Finally, the Thomson scattered component may account for the off-pulse emission of Her X-1 which has a spectrum similar to that of the pulsed component. Another source of the diffuse off-pulse component may be the scattering by surface micro-roughness or Bragg reflection by the one-dimensional lattice of the magnetic polymer constituting the surface matter. A more detailed model for the complex spectral variability of Her X-1 will be discussed in a separate paper.

After writing this paper we became aware of ref. 13, which contains an alternative and more general treatment of the electrodynamical problem of 'reflection and refraction of electromagnetic waves on plasma in homogeneous magnetic fields'. This problem has also been discussed in ref. 14 in the context of emissivity of magnetic neutron star surfaces.

Received 8 August; accepted 3 November 1977.

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Rb-Sr mantle isochrons and variations in the chemistry of Gondwanaland's lithosphere

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Isotopic data for the Mesozoic tholeiites of Gondwanaland are used to evaluate theories explaining the chemistry of mantle-derived rocks; a crustal contamination hypothesis is discarded. These findings may contribute towards an understanding of crust-mantle evolution.

BECAUSE of the potential importance of Rb-Sr mantle isochrons^{1,2} in models of igneous petrogenesis, we have examined the published isotopic data for the Mesozoic tholeiites of Gondwanaland. These tholeiites were chosen because they are the product of roughly synchronous magmatism distributed over a vast surface area and they share a similar mode of occurrence and major element chemistry. Thus they can be used to appraise the question of primary versus contaminated chemistry in mantle-derived rocks and also the possibility that plateau-type tholeiites contain information on the local and regional heterogeneities in Earth's mantle.

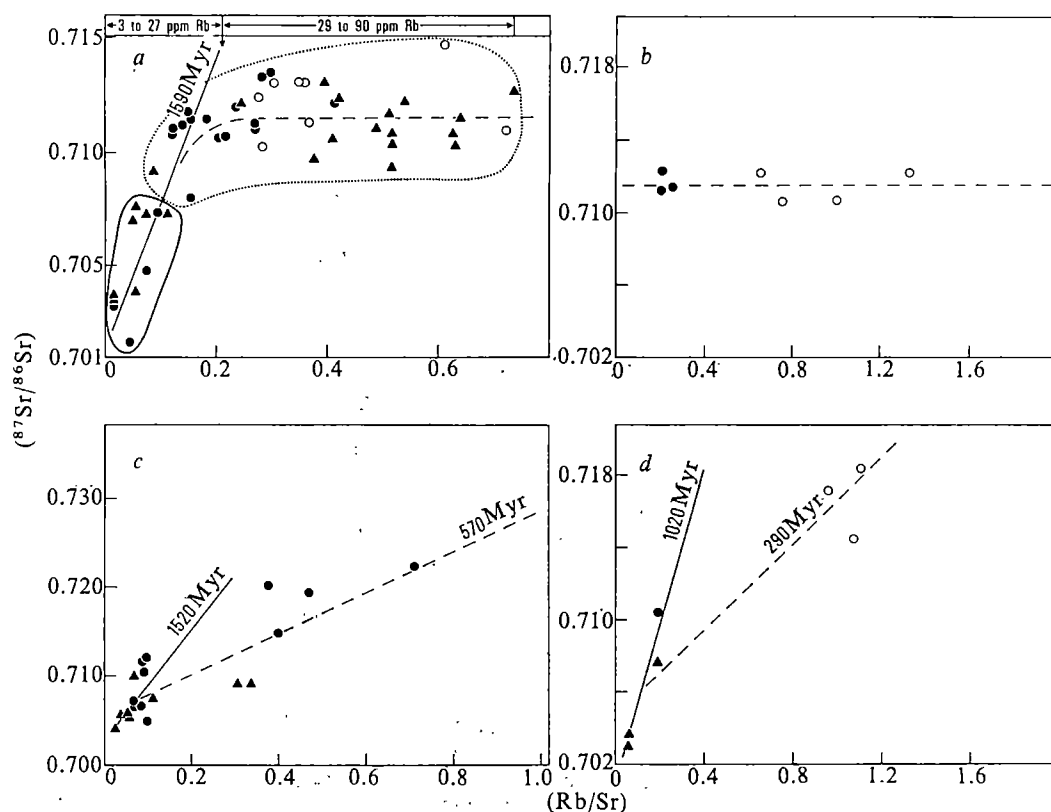
Mantle isochron properties

All available Rb/Sr and Sr-isotopic data for these tholeiites have been retrieved from the literature, grouped according to geographic distribution both within and between the individual post-Gondwanaland continental fragments, and examined for Rb-Sr mantle isochron properties. We have corrected the retrieved ⁸⁷Sr/⁸⁶Sr ratios for their post-crystallisation component by applying a blanket 160 Myr age correction. The Rb/Sr of the samples is such that real variation in this correction age has little influence on the calculated initial ⁸⁷Sr/⁸⁶Sr values. The resulting initial ⁸⁷Sr/⁸⁶Sr versus Rb/Sr data for each association are presented in Fig. 1. Each plot includes the more felsic members of each association (for example, the pegmatoids of the Ferrar Group; the Rio Grande do Sol felsics of the Serra Geral Formation; the Red Hill granophyres of Tasmania). Inspection of the diagrams reveals that good correlations occur for three of the five associations (Karoo, Serra Geral

and Queen Maud Land dolerites) while the remaining two associations (Ferrar Group and Tasmanian dolerites) define horizontal lines corresponding to a small range in initial ⁸⁷Sr/⁸⁶Sr for a wide range in Rb/Sr. Furthermore, for two of those associations showing correlations, rejection of the felsic members with high Rb/Sr leads to a correlation which, if interpreted as an isochron, corresponds to a much older age (Fig. 1c, d). The relevant statistical data for these correlations together with literature sources and number of samples are given in Table 1; the resulting best-fit lines are significantly different from zero at levels of confidence usually greater than 95%.

There are a variety of mechanisms which could produce correlations of the type observed in Fig. 1 and no obvious criteria to permit distinction between a crustal and mantle-related process. There is, however, considerable evidence against crustal contamination as the cause of these correlations. For the Mesozoic tholeiites such evidence would include lack of 'mixing line' relationships between all of the elements analysed, despite the thesis that the variability in isotopic composition is due to variable assimilation of that contaminant. This is well illustrated for the Kirkpatrick Basalts of Antarctica³, the proposed effusive phase of the Ferrar dolerites⁴. These basalts show initial Sr versus major element correlations for SiO₂ and total Fe, and a single contaminant is advanced to explain this correlation. However, the Sr-isotopic composition does not correlate with many of the other major elements (including Na and K) nor with Rb. The latter is higher in concentration than levels normally observed in basaltic magmas and any mixing-contamination explanations must satisfy the Rb observations. Other evidence would be an unlikely composition of supposed contaminant; any reasonable contamination mechanism will probably involve more than one rock composition; however, the average contaminant must correspond to some reasonable rock not too different in composition from that of the average crust. For the Kirkpatrick basalts, the proposed contaminant had a CIPW norm composition of:

Fig. 1 Rb/Sr versus initial $^{87}\text{Sr}/^{86}\text{Sr}$ for Mesozoic tholeiites. ●, Dolerites; ▲, basalts; ○, felsic members (such as dacites, granophyres); solid line mantle isochrons are for the less differentiated samples ($\text{Rb}/\text{Sr} < 0.2$, that is, $\text{SiO}_2 < 53\%$) and the dashed-line mantle isochrons are for all the data from each association (including felsic members if available). For literature sources see Table 1. *a*, Antarctica: Ferrar Group (including Kirkpatrick basalts) enclosed in dotted-line field; Queen Maud Land dolerites enclosed in solid-line field. The range of Rb values (p.p.m.) is included for reference. The solid-line mantle isochron is for the Queen Maud Land samples only. *b*, Australia: Tasmanian dolerites and granophyres. *c*, South Africa: Karroo dolerites and basalts (including samples from the Lebombo Nuanetsi Igneous Province). *d*, South America: Serra Geral Formation.



ferrosilite, 28%; quartz, 25%; albite, 19%; orthoclase, 18%; Na-metasilicate, 4%; diopside, 3%; ilmenite, 1%; apatite, 1%. While it is possible that mixtures of some crustal assemblages could produce a contaminant of this composition, it is highly unlikely that such a contaminant could exist in sufficient quantities to account for the wholesale contamination required by the model. Similar conclusions were reached by Weigand and Ragland⁵ in discussing the origin of the Mesozoic tholeiites of eastern North America.

The many other similar arguments would not strengthen our case against crustal contamination, but rather dilute it in prolixity. We thus use the isotopic data for the Mesozoic tholeiites to render the crustal contamination hypothesis untenable.

Isochrons for oceanic tholeiites

In oceanic regions, remote from contamination by continental crust, correlations of the type shown in Fig. 1 often occur².

Table 1 Rb-Sr mantle isochron data for Mesozoic tholeiite associations of Gondwanaland

Location	Association	MIRA	MIIR	<i>r</i>	<i>N</i>	Reference
ZONE A						
South America	Serra Geral Formation: all data (<i>b,d,d¹</i>)	290 ± 50	0.7047 ± 14	0.93(3)	7	9
	data with $\text{Rb}/\text{Sr} < 0.2$ (<i>b,d</i>)	1,020 ± 280	0.7011 ± 16	0.93(4)	4	9
South Africa	Karoo System: all data (<i>b,d</i>)	570 ± 90	0.7059 ± 10	0.56(2)	19(2)	25, 27, 28
	data with $\text{Rb}/\text{Sr} < 0.2$ (<i>b,d</i>)	1,520 ± 640	0.7031 ± 20	0.84(4)	13	25, 27, 28
Antarctica	Queen Maud Land: all data (<i>b,d</i>)	1,010 ± 420	0.7031 ± 11	0.63(1)	11(1)	4
ZONE B						
Antarctica	Ferrar Group: all data (<i>b,d,g,p</i>)	n.s.	n.s.	n.s.	40	25, 4, 3
	data with $\text{Rb}/\text{Sr} < 0.2$ (<i>b,d</i>)	n.s.	n.s.	n.s.	8	25, 4, 3
Australia	Tasmanian dolerites: all data (<i>d,g</i>)	n.s.	n.s.	n.s.	7	26
THOLEIITES						
	Gondwanaland average data plus oceanic tholeiites, excluding Serra Geral value	1,620 ± 55	0.7023 ± 1	0.98(4)	14	2, and above list

All ± values given at 1σ. Details of mantle isochron regression procedures given in ref. 1. MIRA, mantle isochron regression age (Myr); MIIR, mantle isochron initial ratio; *r*, Pearson correlation coefficient (the numbers in parenthesis are increments of the standard deviation and indicate the level of confidence at which the slope of the fitted regression line differs from zero); *N*, samples included in the regression (the numbers in parenthesis are the numbers of available samples excluded); *b*, basalt; *d*, dolerite; *d¹*, dacite or rhyodacite; *g*, granophyre; *p*, pegmatoid; n.s., not significant. All averaged data from Table 2.

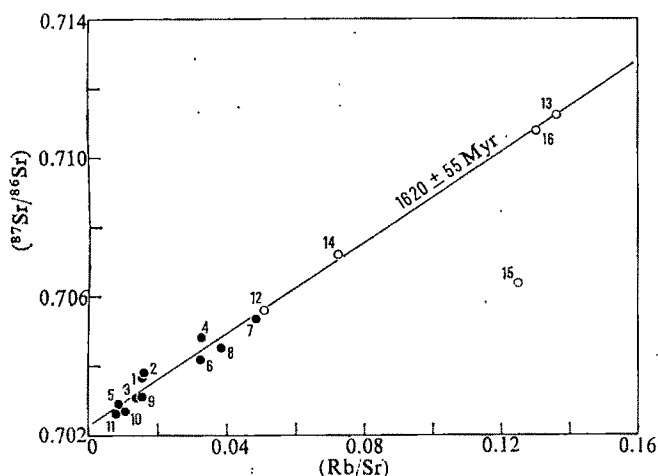


Fig. 2 Rb-Sr mantle isochron (solid line) for oceanic and Mesozoic tholeiites. Each point represents an average of available data on individual samples from either a given island, island group (●) or continental association (○). Numbers refer to the data index in Table 2.

For average island tholeiites these correlations correspond to an age of $1,600 \pm 200$ Myr, which is remarkably concordant with the Pb-Pb isochron ages of oceanic basalts ($1,800 \pm 100$ Myr). Because of this concordance between radiometric systems in which the systematics differ markedly during petrogenesis (especially during variable degrees of partial melting and differentiation), we argued that the Rb/Sr data defined a mantle isochron dating a real event, possibly the separation of an isolated asthenosphere (and mesosphere) within a previously more uniform and convecting mantle. In arriving at the 1,600 Myr Rb-Sr mantle isochron for oceanic tholeiites, we averaged the data from each island or island group, excluding any differentiate samples which could have influenced the systematics. The same treatment has been applied to the Mesozoic tholeiites, where differentiation has played a significant part in their magmatic histories as revealed by major element compositions (for example, refs 6, 7). Because only a few isotopic analyses have accompanying major and trace element data we could not independently identify the more primitive samples. Examination of available major element, trace element and petrographic data, however, indicate that the Rb/Sr ratio is extremely sensitive to bulk chemistry and can be used in place of a major or trace element index of differentiation. It was found that a Rb/Sr ratio of about 0.2 corresponds to an SiO_2 level of approximately 53%, and hence samples with an Rb/Sr ratio of 0.2 or less have chemical compositions more like 'normal' basaltic tholeiite. It is recognised that samples with Rb/Sr less than this value could also be somewhat differentiated and we emphasise that the Rb/Sr cut-off of 0.2 is used merely to distinguish between the more and less differentiated tholeiites. The effects of this differentiation on the mantle isochron systematics is well illustrated by the Karroo and Serra Geral tholeiites. Rejection of samples with Rb/Sr > 0.2 leads to significantly older mantle isochrons in the range of 1,000 to 1,500 Myr (Fig. 1 and Table 1). Clearly, differentiation rotates the mantle isochrons to younger ages, thereby masking source-region age information.

The calculated average values for initial $^{87}\text{Sr}/^{86}\text{Sr}$ and Rb/Sr of the least differentiated samples from each association are listed with the modern oceanic tholeiite data in Table 2 and plotted in Fig. 2. The Mesozoic data plot along the extension of the oceanic tholeiite mantle isochron with the exception of the Serra Geral Formation, where weathering may have rendered the data suspect. Published data for the Tasmanian samples all have Rb/Sr > 0.2, and so were excluded from this plot, but unpublished data with Rb/Sr < 0.2 have been included in Fig. 2 (these data will be the subject of a separate paper). When treated together, the tholeiite data define a 1,620 Myr mantle isochron (Table 1) with a 1σ error

Table 2 Average Rb/Sr and initial $^{87}\text{Sr}/^{86}\text{Sr}$ for tholeiites from oceanic islands[†], ocean floors[†] and the Gondwanaland Mesozoic tholeiite associations (this paper)

Location	Rb/Sr	$^{87}\text{Sr}/^{86}\text{Sr}^*$	N^\dagger	Graph no. [‡]
Oceanic island tholeiites				
Bouvet	0.016	0.70369	4	1
Hawaiian Islands	0.017	0.7038	30	2
Iceland	0.014	0.70307	13	3
Kerguelen	0.033	0.7048	5	4
Kolbeinsey	0.009	0.70290	1	5
Reunion	0.033	0.7042	8	6
Samoa	0.049	0.7053	5	7
St Pauls	0.039	0.7045	7	8
Ocean floor tholeiites				
Indian	0.015	0.70314	8	9
Pacific	0.011	0.70265	15	10
Atlantic	0.008	0.70264	13	11
Gondwanaland tholeiites				
Queen Maud Land	0.051	0.7056	11(1)	12
Ferrar Group	0.136	0.7112	7(1)	13
Karoo System	0.072	0.7072	13	14
Serra Geral Fm.	0.125	0.7063	4	15
Tasmania	0.130	0.7108	4	16

*Relative to *E* and *A* standard value of 0.70800.

[†] N = number of samples averaged, value in parenthesis is number of available samples excluded from the average.

[‡]Reference number for Fig. 2.

Average data for least differentiated tholeiites (Rb/Sr < 0.2).

(± 55 Myr) comparable to that of geochronological-type isochrons used for standard stratigraphic purposes. This alignment of oceanic island and Mesozoic tholeiite data along a common isochron would seem to require an exceptional coincidence if crustal contamination is the reason for the high $^{87}\text{Sr}/^{86}\text{Sr}$ ratios in the Mesozoic tholeiites. We consider this to be significant evidence against crustal contamination as a cause for the elevated and variable Sr isotope ratios in these tholeiites. It is also contradicts previous arguments for the bulk assimilation of crustal material in their petrogenesis, and encourages the interpretation of the isotope compositions of these rocks in terms of primary mantle properties.

Gondwanaland regional variations

When the five associations studied in this paper are plotted on a reconstructed Gondwanaland⁸, they define two geographic

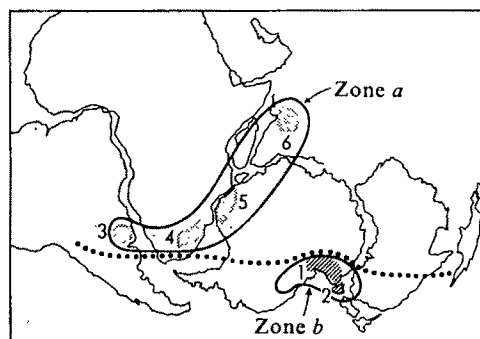


Fig. 3 Location of Mesozoic tholeiite associations plotted on Smith and Hallam's⁸ reconstruction of Gondwanaland. The continental interior margin of Du Toit's²⁴ Samfrau geosyncline is shown (heavily dotted line). Zone *a* includes Mesozoic tholeiites that have traversed stable cratonic interiors of the continents while Zone *b* includes those tholeiites that have traversed cratonic-margin regions. 1, Ferrar Group including the Kirkpatrick basalts; 2, Tasmanian dolerites of south-east Australia; 3, Serra Geral Formation, South America; 4, Karroo dolerites including the Lebombo-Naunetsi Igneous Province, South Africa; 5, Queen Maud Land dolerites of Antarctica; 6, Deccan Traps, India.

provinces (labelled zones *a* and *b*, Fig 3). These two provinces differ in several ways. Zone *a* tholeiites have traversed ancient basement rocks (2,000 Myr or older; refs. 9–13) constituting the cratons of each post-Gondwanaland fragment, while zone *b* tholeiites have been emplaced in a cratonic margin region dominated by late Precambrian and Palaeozoic orogenesis (basement ages in the range 400 to 1,000 Myr; refs. 14–17). Zone *a* has 'normal' surface heat flux values typical of cratonic regions while zone *b* is characterised by heat flow values about twice those of zone *a*^{18, 19}. Zone *a* tholeiites show mantle isochrons, zone *b* tholeiites do not. Zone *a* tholeiites show lower average ⁸⁷Sr/⁸⁶Sr ratios (0.7064) than zone *b* tholeiites (0.7112).

Considering these differences, we suggest that the lithosphere under zone *b* is significantly more enriched in radioactive heat producing elements than that underlying zone *a*. A relative enrichment in zone *b* lithosphere of K, Rb and U would lead to higher surface heat flow values and higher ⁸⁷Sr/⁸⁶Sr ratios. The lack of mantle isochrons in zone *b* tholeiites may reflect a relatively homogeneous or well-mixed lithosphere in this region. This suggests that zone *b* lithosphere may be composed dominantly of 'enriched' mesosphere-derived material relative to more depleted asthenosphere components. Also, high initial ⁸⁷Sr/⁸⁶Sr ratios (0.71 or more) are pervasive in many mafic igneous rocks that have been emplaced in the Australian sector of the Samfrau Geosyncline^{17, 20} (L. Black, personal communication, C. B., unpublished data) indicating a consistent regional anomaly in the source region corresponding to zone *b*. In Fig. 3, we have extended zone *a* to India because the Mesozoic tholeiites of that sub-continent (Deccan Traps) occur within a craton with basement of at least 2,500 Myr²¹. It could be predicted that, when data become available, the Deccan Traps will also show Rb–Sr mantle isochrons, although whether the accompanying range in initial ⁸⁷Sr/⁸⁶Sr resembles that of the Karroo and Serra Geral tholeiites or that of the Queen Maud Land dolerites awaits investigation. Based on our analysis of the Mesozoic tholeiites of Gondwanaland, a case can be made for the existence of gross heterogeneities in the underlying lithosphere manifest on all scales from local to continental, and for the development of those heterogeneities during an event perhaps associated with the separation of asthenosphere from mesosphere 1,600 to 1,700 Myr ago. Furthermore, we find that the cratonic margin part of Gond-

waland's lithosphere is probably much more enriched on average than the regional sub-cratonic lithosphere. It is not accidental that this enriched lithosphere correlates with areas of high heat flow, numerous anatectic granites^{22, 23}, high incidence of economic mineralisation of diverse types, and anomalous Sr-isotope composition in many mafic igneous rocks of Precambrian to Palaeozoic age. Indeed one may speculate that the reactivation of that sector of Gondwanaland during the late Precambrian and Palaeozoic was due primarily to the enriched nature of the underlying lithosphere. Clearly we have left many questions unresolved and there are many new ones provoked, but we are confident that investigations orientated to searching for and understanding Rb–Sr mantle isochrons will have a profound effect on our understanding of crust–mantle evolution.

This work has been supported by the NRC of Canada, the USNSF and the Harry Oscar Wood Fund.

Received 13 October; accepted 9 November 1977.

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Structure of vancomycin and its complex with acetyl-D-alanyl-D-alanine

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Vancomycin, a broad-spectrum antibiotic, inhibits the growth of cell walls by complex formation with peptides terminating in D-alanyl-D-alanine. The structure of vancomycin was determined by X-ray analysis of the degradation product CDP-I. A model of the complex is proposed based on this study and spectroscopic data.

THE antibiotic, vancomycin, was first isolated in 1956 from cultures of *Streptomyces orientalis*¹. Although much studied; its full chemical structure could not be established. It is a broad-spectrum antibiotic but because of adverse side effects, its use is restricted to the treatment of staphylococcal infections (for example, septicaemia, pneumonia and wound infections) when

other antibiotics are ineffective. There are no naturally occurring strains of bacteria known to be resistant to vancomycin, nor do bacteria readily develop resistance to it. Clinical studies of vancomycin have been reviewed by Lightbown².

Vancomycin is known to act by inhibiting the biosynthesis of the cell-wall mucopeptide, leading to a cessation of growth and the eventual destruction of the cell by lysis. Perkins has shown^{3–5} that it forms complexes with the nucleotide-*N*-acetyl muramyl pentapeptide precursor, the reaction being specific for peptides with D-alanyl-D-alanine at the carboxyl terminus. Such complex formation either with the lipid intermediate or with uncross-linked peptidoglycan would inhibit mucopeptide biosynthesis and thus account for the antibiotic action. A more detailed understanding of this complex formation, however, is not possible without a knowledge of the molecular structure of the antibiotic.

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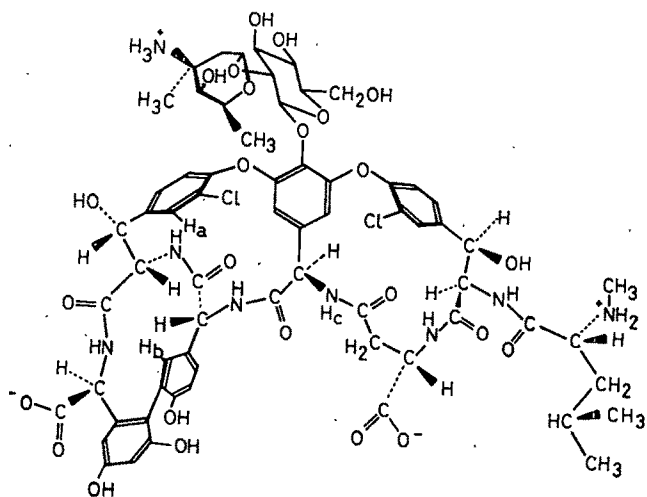


Fig. 1 The structure of CDP-I.

The structure of vancomycin has now been determined from an X-ray analysis of CDP-I⁶, a degradation product obtained from vancomycin with loss of ammonia. The solution of the structure, which contains 101 atoms (excluding hydrogens) in the asymmetric unit as well as 33 disordered water molecules, was obtained by direct methods, with the minimum use of chemical information. The analysis was based on full data out to a resolution of 0.8 Å, and without such extensive data it is unlikely that the structure could have been solved by the techniques available at present.

X-ray analysis of CDP-I confirms the structural units deduced from chemical, nuclear magnetic resonance (NMR), and mass spectrometry experiments⁶⁻¹². The principal units in the structure, summarised in Fig. 1, are a disaccharide linked to three aromatic rings; *N*-methylleucine; aspartic acid and a biphenyl system. These units are linked by secondary amide bonds, to form a tricyclic molecule, containing *N*-terminal *N*-methylleucine and two free carboxyl groups.

Aspects of the conformation of vancomycin in DMSO solution (from NMR data) correlate closely with the three-dimensional structure determined by X rays. There are thus no substantial conformational changes resulting from hydration or crystal forces. We present here details of the X-ray analysis, the three-dimensional structure and some suggestions concerning the mode of binding of acetyl D-alanyl-D-alanine to vancomycin. If suitable crystals can be obtained, we hope to test these speculations by an X-ray analysis of such a complex.

Experimental details

Large crystals of CDP-I were obtained by dissolving vancomycin in water, adjusting the pH to 4.2 with a few drops of 0.05 N aqueous NaOH, and keeping the resulting solution at 70–80 °C for 40 h. During the course of the reaction, large, highly insoluble amber crystals separated out, one of which was cut to a rectangular block 0.7 × 0.3 × 0.2 mm and mounted in a capillary tube in contact with some mother liquor. Cell constants were measured on a 4-circle Syntex diffractometer using Cu Kα radiation (Table 1). The cell dimensions were slightly larger than previously reported¹³, probably because of increased hydration in this sample.

In total, 11,986 reflections, including Friedel pairs, were measured on the diffractometer in the range $0 < 2\theta < 101^\circ$ giving 7,759 unique reflections with $F > 3\sigma(F)$. A repeatedly monitored reference reflection decreased in intensity by ~20% during the 5 weeks of data collection and other intensities were scaled accordingly.

Solution of the structure

Numerous fruitless attempts were made to solve the structure using both Patterson and direct methods. Eventually a chemically sensible fragment, containing a six-membered ring and an atom

Table 1 Crystal data for CDP-I

Formula	$C_{66}H_{76}Cl_2N_8O_{25} \cdot xH_2O$
Space group	$P2_12_12_1$
Z	4
a	13.926(5) Å
b	21.578(5) Å
c	33.802(14) Å
V	10157(10) Å ³
Radiation	Cu Kα, $\lambda = 1.5418$ Å
μ	13 cm ⁻¹

assumed to be chlorine, emerged fortuitously from one of the E-maps. Tangent expansion and guesswork extended this to the three aromatic rings of the central nucleus and the two chlorine atoms. All attempts to expand this fragment, including the use of translation functions, were unsuccessful, until the geometry had been optimised by least-squares refinement with distance constraints. A single tangent expansion then produced 80 atoms, all of which could be identified with the units deduced chemically. Further atoms, including the water molecules, were located from difference syntheses.

The structure has now been refined to a current R of 16%. All atoms except Cl are isotropic. Fifty-four H atoms were included at calculated positions (C–H 1.08 Å, H–C–H 109.5°); 33 water molecules were located, with site occupancy factors varying from 0.47 to 1. The temperature factor for the disordered water molecules was fixed at 0.15 Å². Refinement is continuing. The absolute configuration has not been determined from the X-ray data, but it must be that shown in Fig. 2 from the known stereochemistry of chemically isolated fragments.

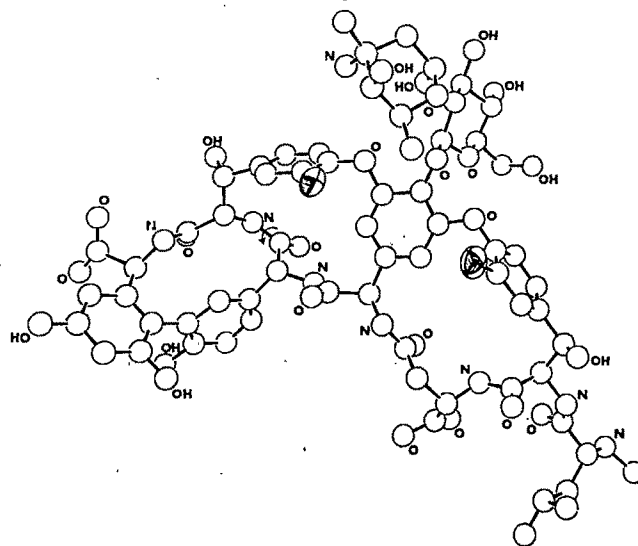
The structure

As already mentioned, all the units deduced from chemical and spectroscopic evidence have been confirmed by the X-ray structure. These include the amino sugar vancomamine, two β -hydroxychlorotyrosine units and three oxygenated phenylglycine systems as well as *N*-methylleucine and aspartic acid. It is unusual that in the latter, the α carbonyl is present as the free carboxyl group. The structure also contains a relatively rare *cis* amide bond.

The molecule is extremely compact, as can be seen from the space filling drawing of Fig. 3. It carries a marked cleft on the side of the molecule bearing the Cl atoms, but also a less well-defined cleft on the other side. There is no space for any water molecules inside the tricyclic ring system.

The molecules are linked in the extended crystal structure by a complex system of hydrogen bonds. There are only five hydrogen

Fig. 2 ORTEP¹⁶ plot of CDP-I. The *cis* amide bond is arrowed; H atoms are omitted. Anisotropic atoms are chlorine.



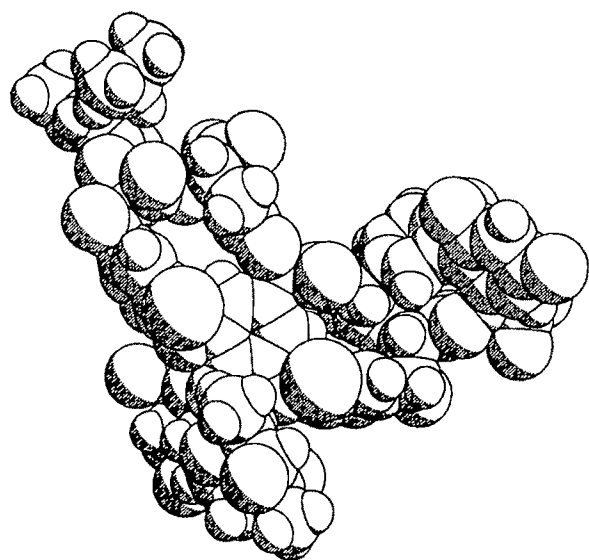


Fig. 3 Space-filling plot of CDP-I (same orientation as Fig. 2, using the program PLUTO, W. D. S. Motherwell, unpublished). The following radii were used (Å): C, N, O all 1.4; Cl 1.7; H 0.9. Only H atoms attached to C are included.

bonds which do not involve water: the carboxyl of aspartic acid to OH of the trisubstituted ring of the biphenyl, NH_2 of methyl-leucine to carboxylate of the biphenyl, O4 of the glucose to NH of the diaryloxyphenyl-glycine and O4 of vancosamine to the carboxylate and NH of aspartic acid. The remaining hydrogen bonds are between the CDP-I molecule and water molecules, and between the water molecules themselves, forming an intricate three-dimensional network.

CDP-I is obtained from vancomycin by the loss of ammonia. The free carboxyl group on the biphenyl group of CDP-I is known also to occur in vancomycin. The structure of vancomycin is therefore that shown in Figs 1 and 2 except that the carboxyl group of the aspartic acid is changed to a primary amide ($-\text{CONH}_2$).

Mode of binding

The molecular basis of the antibiotic action is probably associated with the binding of acyl-D-Ala-D-Ala fragments into the cleft of the molecule on the face bearing the chlorine atoms. Earlier NMR studies^{14,15} have shown that the C-terminal alanine methyl resonance of Ac-D-Ala-D-Ala is heavily shielded in the presence of vancomycin. Later work¹² established that binding of Ac-D-Ala-D-Ala to vancomycin caused marked downfield shifts of protons

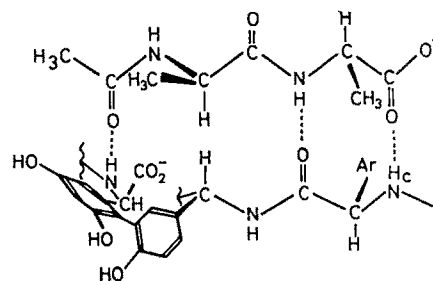


Fig. 4 Schematic illustration of a possible interaction between Ac-D-Ala-D-Ala and a portion of the vancomycin molecule.

H_b and H_c , and a marked upfield shift of H_a (Fig. 1). It is likely that these protons are near to the peptide fragment in the antibiotic-peptide complex, and in particular that NH_c is hydrogen bonded to a peptide carbonyl group. On the basis of this evidence, and an examination of space-filling models, we propose that vancomycin associates with the backbone of Ac-D-Ala-D-Ala as shown in Fig. 4, where the aromatic residue (Ar) is the trioxxygenated benzene ring bearing the disaccharide. In the proposed complex, the C-terminal methyl group of Ac-D-Ala-D-Ala lies above the face of this benzene ring, thus accounting for its upfield shift on complex formation. The other secondary methyl group of Ac-D-Ala-D-Ala lies near the benzene ring which carries two phenolic hydroxyl groups, but is not located centrally over it and so is not similarly shielded on complex formation.

This model, although consistent with the X-ray structure and spectroscopic results, is not necessarily a unique one, and further experimental evidence is needed to establish the precise mode of binding of the antibiotic-peptide complex.

We thank the MRC for financial support and the SRC for the provision of the diffractometer.

Received 13 October; accepted 9 November 1977.

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letters to nature

Positions of galactic

X-ray sources: $320^\circ < l^{\text{II}} < 340^\circ$

PRECISE ($20-40''$) positions of seven X-ray sources in the celestial region $320^\circ < l^{\text{II}} < 340^\circ$ are reported here. These include a recurrent transient X-ray source (MX1608-52; refs 1-4) and a source 2S1553-542 (ref. 5) coincident with a γ -ray source⁶ within the given errors of position. The positions reported here reduce the previously reported areas

of the error region for six of the sources by factors ranging between 10 and 100. In the case of MX1608-52, a preliminary report of this work⁷ led to the identification of an optical candidate^{7,8}. The present results add confidence to the proposed radio candidates for 4U1624-49 (ref. 9) and 4U1642-45 (refs 9, 10). But they eliminate proposed possible optical candidates^{5,11-14} for the sources 4U1543-62, 2S1553-542, 4U1624-49 and 4U1642-45.

The positions for the sources 4U1538-52, 4U1543-62, 2S1553-542, (MX1553-54), 4U1556-60, MX1608-52,

Table 1 Celestial positions

SAS-3 designation	Other designations*	Position (1950) α	δ	μ b^{11}	Error radius (90%)	Flux density† (2–11 keV)	Comments
2S1538–522	4U1538–52	15h38m40.2s 234.6675°	–52°13'38'' –52.2272°	327.42° 2.16°	40''	9 μ Jy	Pulsing binary ^{23, 25, 26}
2S1543–624	4U1543–62	15 43 33.9 235.8912	–62 24 56 –62.4155	321.76 –6.34	30	38	
2S1553–542	MX1553–54	15 53 55.6 238.4817	–54 16 15 –54.2708	327.95 –0.86	35	27	γ -ray source CG327–0? (ref. 6)
2S1556–605	4U1556–60	15 56 47.2 239.1967	–60 35 47 –60.5963	324.15 –5.93	30	16	
2S1608–523	MX1608–52 4U1608–52	16 08 51.2 242.2133	–52 18 02 –52.3005	330.93 –0.85	20	618†	Recurrent transient ^{2–4} Burst source? ^{30, 31} Optical candidate? ^{7, 8} Radio candidate ⁹
2S1624–490	4U1624–49	16 24 19.6 246.0817	–49 05 07 –49.0853	334.92 –0.26	35	49	
2S1642–455	4U1642–45 GX340+0	16 42 09.9 250.5412	–45 31 10 –45.5194	339.59 –0.08	30	338	Radio candidate ^{9, 10}

*Refs 1, 5, 20, 32.

†1.0 μ Jy corresponds to 2.2×10^{-11} erg s^{–1} cm^{–2} (2–11 keV). $I_{\text{crab}} = 1,060$ μ Jy (see refs 15, 16).

‡At the time of observation (24 July, 1977).

4U1624–49, and GX340+0 (4U1642–45) are reported in Table 1 along with the estimated error radii¹⁵. The observations of six of these sources were carried out with the SAS-3 rotation modulation collimator from 16 June to 21 June 1975 as part of a survey of galactic source positions^{15–19}. The source MX1608–52 was observed on 24 July 1977 when it was in a bright state². In Fig. 1, we compare our results with other measurements and positions of radio and optical candidates for three of the sources. Finding charts for six sources are given in Fig. 2. Astrometry and photometry results for a few stars in the error regions are given in Table 2.

Ten other known X-ray sources lie in this region. SAS-3 positions for three of these (Cir X–1, TrA X–1 = A1524–61, MXB1636–53) were reported earlier^{17, 18}. The remaining seven were not seen in our survey. Of these, the sources 4U1543–47 and 4U1630–47 are known transients^{20, 21} and the source 4U1658–48 is known to be highly variable²². We place an upper limit of 10 μ Jy (2–11 keV) for these and the other four sources (4U1510–59, 4U1530–44, A1540–53 and 4U1631–64). The intensity of MX1608–52

was ≤ 5 μ Jy during the 1975 observations. These limits are consistent with the intensities and variability factors previously reported for these sources^{20, 22}.

We discuss briefly below the proposed radio and optical candidates for the sources:

4U1538–52: Cowley *et al.*²⁴ have measured the colours for ~ 50 stars in the Uhuru error region and have suggested several probable candidates for this source; stars numbered 10 and 12 in Fig. 2 are among these. This source is now known to be the same as A1540–53 (ref. 23), on the basis of new Ariel V position determinations²⁵ and is also known to be a pulsing binary system^{23, 25, 26}.

4U1543–62: The star suggested as a possible candidate by Penston *et al.*¹¹ (denoted as A in their Plate II) is excluded as a candidate.

2S1553–542: The variable star TT Normae and the B0 supergiant SAO243166 are near our error circle (Fig. 1). We previously suggested⁵ the latter as a candidate. Subsequently, failure to detect emission lines or velocity variations in its spectrum²⁷ weakened the proposed identification. This star is $\sim 1.2'$ away from the refined position given here and

Fig. 1 X-ray source positions and candidates for three sources. The SAS-3 error circles for the other sources are smaller in area than previous work by factors of 10–100. The positions are from Uhuru (U, ref. 20), and Ariel V (A, ref. 33) satellites, a rocket experiment³⁴, and the present work (S). The stars TT Normae and SAO243166 near 2S1553–542 and the star SAO226781 near 2S1624–490 are also shown. The position of the radio candidate⁹ for 4U1624–49 and the extent of a finite-sized radio source^{9, 10} coincident with 4U1642–45 are indicated.

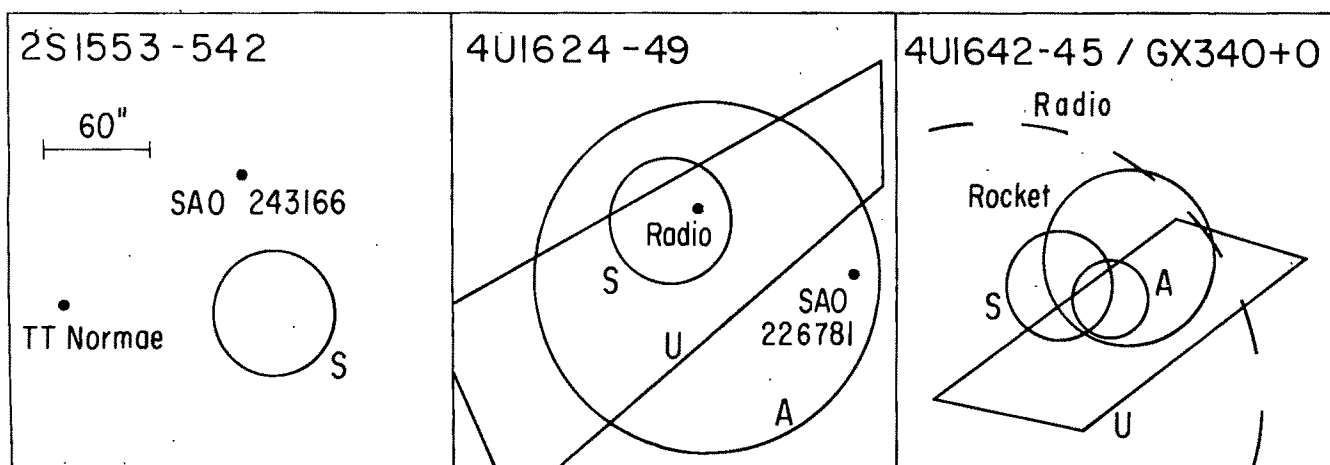


Figure 1

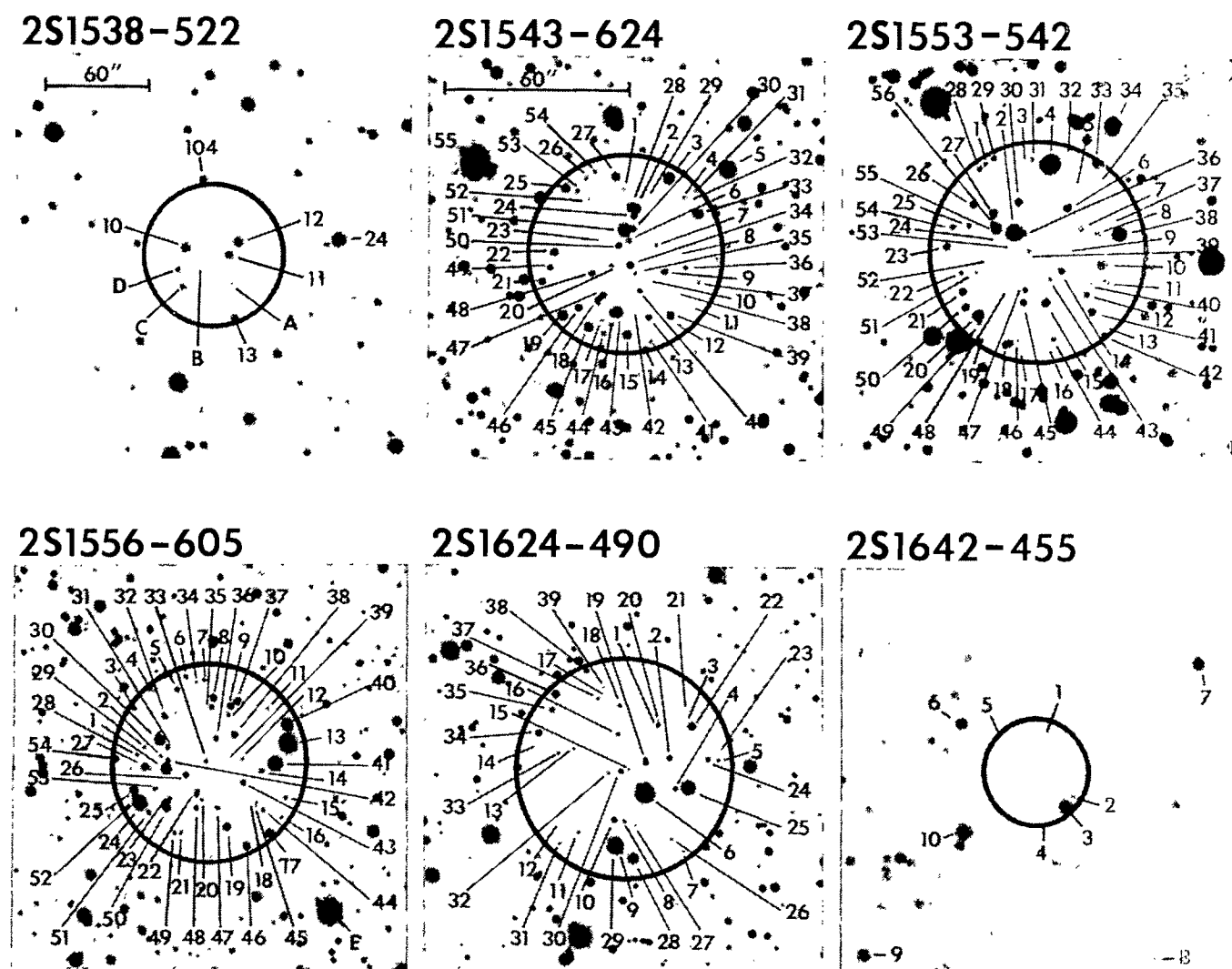


Fig. 2 Finding charts for six SAS-3 positions. A finding chart for 2S1608-523 is given by Grindley and Liller⁸. The charts for 2S1553-542 and 2S1624-490 are from R plates and those for 2S1543-624 and 2S1556-605 from B plates taken with the CTIO telescope by W. A. Hiltner and C. Canizares; see chart 2S1543-624 for scale. The other two charts are from the ESO quick-blue survey; see 2S1538-522 for scale. We adopted the numbering scheme of ref. 24 for 2S1538-522 and the lettering scheme of ref. 11 for 2S1556-605. North is up and east is to the left.

is excluded as a possible candidate. This source is within the 1° radius error circle of the γ -ray source CG327-0 discovered by the COS-B satellite⁶.

4U1556-60: The star¹⁴ SAO253382 excluded as a candidate by the Copernicus satellite measurement^{11,28}, is also excluded by the present observation.

MX1608-52: The star SAO243445 within the Uhuru error box²⁹ (denoted as star A in Fig. 1 of ref. 29) is excluded by the present measurement. Grindley^{7,8} reports a candidate star within our error circle, which brightened from $V > 23$ mag in August 1976 to $V = 21 \pm 1$ mag in August 1977. The present position is within the error region of the Norma X-ray burst source^{30,31}.

4U1624-49: The radio source G334.9-0.3 proposed by Sanduleak and Dolan⁹ as a candidate is supported by the present measurement (Fig. 1). The star SAO226781 considered as a possible candidate¹¹⁻¹⁴ is excluded by the present observation.

4U1642-45: The extended radio source G339.6-0.1 proposed by Sanduleak and Dolan⁹ and by Seaquist¹⁰ as a candidate is supported by the present observation (Fig. 1). Seaquist¹⁰, on the basis of the flat radio spectrum, suggests that the radio source is an HII region. The star suggested as a possible candidate by Penston *et al.*¹¹ (denoted as A in their plate III) is excluded by the present measurement.

We thank the staffs of the Center for Space Research at MIT and the Goddard Space Flight Center of NASA for their support. We thank C. Canizares, R. Kelly, J. Kulik, J. McClintock, E. Ng, and C. Reid for helpful conversations. This work was supported in part by the US National

Table 2 Stellar astrometry and magnitudes					
Source	Star	m_v^*	Position (1950) [†]		
			α	δ	
2S1538-522	24	13.7 [‡]	15h 38m 32.3s	-52° 13' 34.8"	
2S1543-624	5		15 43 29.1	-62 24 27.1	
	3	15.3			
	55		15 43 41.2	-62 24 25.1	
2S1553-542	34		15 53 52.8	-54 15 30.8	
	2	15.0			
	4	13.8			
2S1556-605	E	11.9 [§]	15 56 42.0	-60 36 32.2	
	13	13.3			
2S1624-490	6	12.6	16 24 18.7	-49 05 13.8	
	28	15.9			
2S1642-455	3		16 42 08.2	-45 31 27.7	
	6		16 42 13.9	-45 30 42.7	

*From photoelectric photometry by J. E. McClintock accurate to 0.1 mag.
[†]Precise to $\leq 3''$.
[‡]From ref. 24.
[§]From ref. 11.

Aeronautics and Space Administration. This is the sixth in a series of articles on positions of X-ray sources obtained with SAS-3.

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Received 17 October; accepted 15 November 1977.

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Dynamic measurement of matter creation

If a rotor could be spun so that it had an inertial decay time $\sim 10^{11}$ yr, 10^{18} s, it could test matter creation cosmologies¹⁻³ at a significant level. The advantage of this method over previous ones⁴⁻⁸ is that the result is independent of the form in which matter is created, providing it stays in place. We have designed an experiment to test this process; it is under construction and is described here. It consists of an inertially spinning rotor suspended inside another rotor, a modification of the double magnetic suspension⁸. Past measurements or estimates have not unequivocally answered the question of matter creation. Cohen and King⁴ established an upper limit of 4×10^{-23} g per g per s on the creation rate of hydrogen in mercury metal. Gittus⁵ suggested that matter created in rocks takes the form of interstitial atoms which diffuse into dislocations. Towe⁶ disputed this, noting that if mass proportional creation were occurring, 30% of the atoms in old terrestrial rocks would occupy interstitial positions and this would result in lattice disruptions. Gittus⁷ later proposed that the matter multiplication rate for crystalline materials could be found by determining the ratio of the shear modulus to the crystal viscosity. He suggested that a reasonable estimate for the sensitivity of the method might be 3×10^{-18} atoms per atom per s, although this has not been tested. In a geophysical argument, Wesson⁸ can account for

the observed expansion rate of the earth by appealing to a mass proportional matter creation whose rate is 7×10^{-18} g per g per s, but he notes that this is in conflict with the result of Cohen and King; it exceeds Dirac's prediction by several times.

Figure 1 shows the elements of the experiment. Two cylinders of highly temperature-stable ceramic rotate concentrically about the axis of rotation of a precision turntable. The outer cylinder is connected to the turntable which rotates with an integral speed constancy limited only by the precision of the atomic clock. The turntable drive is a 50-pole magnetic-averaging synchronous direct drive and the sensor is a double-plate totally averaging encoder system.

The inner (inertial) cylinder is magnetically suspended from the outer and initially caused to rotate with the outer, after which it is freely rotating. Mass created in the inner cylinder causes it to slow down relative to the outer cylinder. The accumulated phase lag of the inertial cylinder provides an extremely sensitive measure of this relative motion. A synchronised pulsed laser has a split beam, part of which is for sensing the relative angle of the two cylinders and another part of which, when unblocked, provides forward or backward momentum impulses to the inertial cylinder to keep it in phase. The sequences of the pulses constitutes the signal. Before and after experiments calibrate the momentum impulses and also provide estimates of limits on competing energy loss mechanisms when the inertial cylinder is driven up to speed from rest with the turntable stationary.

Three major competing mechanisms for energy loss among many^{9,10} are (1) viscous losses in the residual gas of the vacuum, (2) magnetic hysteresis in the support, and (3) interaction with external magnetic fields. Another consideration is the change in cylinder radius due to thermal expansion, and a further major factor is the wandering of the inertial cylinder about its equilibrium position due to thermal noise.

The viscous losses and magnetic hysteresis are rendered virtually zero by the feedback system described above which allows essentially no relative motion between the cylinders. Shields can reduce asymmetric (hysteresis) effects of external magnetic fields to insignificant levels. Thermal expansion effects are kept negligible if the system is thermally isolated and allowed to come to temperature equilibrium, $\Delta T \leq 10^{-3}$ K and by making the cylinder of a material with temperature coefficient $\alpha < 10^{-7}$ K⁻¹. In this case $\Delta r/r < 10^{-10}$ and constitutes a short term variation only, not a steady drift.

The thermal fluctuations can be estimated from standard noise criteria:¹¹

$$\frac{1}{2} I (\Delta \omega_n)^2 = -\frac{1}{2} kT, \quad (1)$$

where I is the inner cylinder moment of inertia, kT is the thermal energy and $\Delta \omega_n$ is the thermally caused fluctuation in velocity.

Then if

$$\Delta \theta_n = \int \Delta \omega_n dt, \quad (2)$$

is the angle through which the cylinder has random-walked from synchronous position due to noise, we get

$$\Delta \theta_n = (kT/I)^{1/2} \tau, \quad (3)$$

where τ is the duration of the experiment.

The magnitude of the expected change in angle due to mass creation depends on the particular cosmology by which it is predicted. If the newly created matter has the same velocity as the matter in the inertial cylinder, then no effect might be measured even if mass creation existed. But as the newly created matter presumably owes its existence in some way to the Universe as a whole, we might expect its initial state to reflect the overall rotation of the Universe which we presume to be zero. In this case, there would be some 'drag' on the inner cylinder.

There are still many possibilities, three of which we will mention. First, angular momentum, L , is conserved and mass

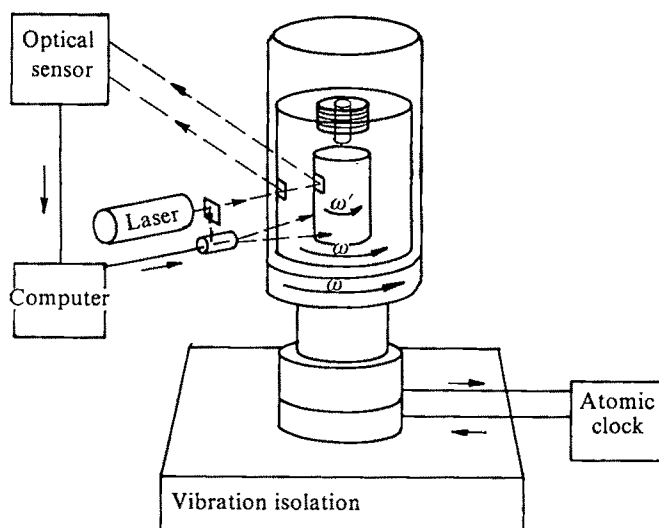


Fig. 1 Two cylinders of temperature-stable ceramic (Zerodur) rotate concentrically in an evacuated region inside an acoustic and magnetic shield. The inner cylinder is magnetically suspended from the outer one which rotates with precise angular velocity ω . Mass created in the inner cylinder tends to slow it down. A feedback system employing laser pulse sensing and photon driving keeps the inner cylinder velocity ω' very near to ω . Forward-backward asymmetry needed in these feedback driving pulses to keep $\omega' = \omega$ constitutes the signal. With the two cylinders running synchronously, viscous, magnetic hysteresis and other damping effects are kept near zero.

is created with no angular momentum and in such a way that the dimensions of the cylinder remain constant. If I = moment of inertia, M = mass, and r = radius of the cylinder, then L = constant = $I\omega \sim Mr^2\omega$ which gives $\dot{\omega}/\omega = -\dot{M}/M$. Second, L conserved; mass created with no angular momentum and density is conserved. As the arguments are dimensional, we will treat the height of the cylinder as the radius, then L = constant = $\rho^{-2/3} M^{5/3} \omega$ which gives $\dot{\omega}/\omega = -5/3 \dot{M}/M$.

Third, large numbers hypothesis as for instance in ref. 1. Under the LNH angular momentum is not conserved in atomic units. Velocity and atomic quantities are constant. If the new matter is created such that density, which is an 'atomic' property is constant, then $\omega \sim v/r \sim v(\rho/M)^{1/3}$, or $\dot{\omega}/\omega = -1/3 \dot{M}/M$. (It is possible to analyse a rotor in more than one way under the LNH; for example, the spinning earth is gravitationally bound and, hence, its density is not purely an atomic property. In this case the analysis might proceed more along the lines of an orbit problem and one gets $\dot{\omega}/\omega \sim -1/2 \dot{M}/M$).

In all of these cases we find $\dot{\omega}/\omega = Y\dot{M}/M$ where Y is a number of order unity. Typical estimates for \dot{M}/M are $2 \times 10^{-10} \text{ yr}^{-1} = 6 \times 10^{-18} \text{ s}^{-1}$. If the inner cylinder has no initial motion relative to the outer, then after a time τ we would have an angular displacement

$$\delta\theta = \omega\tau^2 = -Y\omega\dot{M}/M \tau^2. \quad (4)$$

Thus, the lag angle increases quadratically in time. Equations (3) and (4) can be combined to give the signal-to-noise ratio $\delta\theta/\Delta\theta_n$, from which the sensitivity to mass creation is

$$\dot{M}/M = [(\delta\theta/\Delta\theta_n) (kT/I)^{1/2}] / Y\omega\tau \quad (5)$$

Without feedback, the sensitivity from equation (5) at room temperature, with $I = 25,000 \text{ g cm}^2$ and $\omega = 10 \text{ rad s}^{-1}$ would be $\dot{M}/M = 10^{-8}$. Feedback if noiseless and of gain G can reduce the effective temperature¹¹ by a factor of $\sim (G)^{1/2}$, and the experiment ultimately will be cooled as well. Appropriate feedback will be controlled by a digital processor. Thus the signal-to-noise condition can be met simultaneously with the

low damping requirement. To emphasise the level of difficulty of the experiment we note, from the secular decrease in the earth's rotational angular velocity¹², largely due to tidal friction, that the earth itself as a rotor is too dissipative by a factor of 20. Braginsky¹³ has shown from noise considerations and fundamental limits on detection sensitivity that mechanical oscillators can in principle have decay times $\sim 10^{-18} \text{ s}$. Similar limits apply to rotors and estimates with our parameters are that this can even be improved.

Ultimately, an observed effect would always be suspected of unaccounted-for-damping, yet most damping effects are expected to be linear in time to a close approximation and the signal is quadratic so that it has a distinctive signature. Nevertheless, if an effect is observed the experiment will be repeated at different initial angles, with different cylinders, and at different temperatures.

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Received 25 July; accepted 16 November 1977.

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Identification of interstellar polysaccharides and related hydrocarbons

INFRARED transmittance spectra of several polysaccharides which are suitably calibrated for comparison with astronomical data have been obtained. We show here that a mean 2.5-15 μm spectrum computed from our measurements is remarkably close to that required to explain a wide range of astronomical data, except for two significant points of departure. The required relative opacity at the 3 μm absorption dip is a factor ~ 1.5 lower than we found in our laboratory measurements: this difference may arise from the presence of water ($\sim 10\%$) associated with terrestrial polysaccharide samples. In the 9.5-12 μm waveband an additional source of opacity seems to be necessary. The close agreement between the spectrum of this excess opacity and the absorption spectrum of propene C_3H_6 points strongly to the identification of hydrocarbons of this type which may be associated with polysaccharide grains in interstellar space.

The presence of interstellar polysaccharides has been deduced¹ by comparing infrared spectra of several galactic infrared sources in the 2-30 μm waveband with model calculations based on transmittance data for cellulose. We show here that the transmittance properties of cotton cellulose gives a good agreement to the observed emission from the Trapezium nebula in the 8-30 μm waveband. But with the adoption of more stringent standards of comparison between observations and theory, we thought it appropriate to 'optimise' the transmittance values over the waveband 2.1-13 μm without departing from the original cotton cellulose data by more than a reasonable margin. In the absence of properly calibrated transmittance data for other polysaccharides we have provisionally assigned our optimal values to a 'notional' interstellar polysaccharide ensemble. It is, therefore, desirable to obtain

experimentally a mean transmittance curve for several related polysaccharides.

Four polysaccharides: α -cellulose (α -cellulose fibre ~99.5% pure), amylose (from potato), arabinogalactan (from Larch wood) and mannan (from bakers yeast) were included in the present study. Samples supplied by Sigma London Chemical Company Limited were dispersed in KBr disks at concentrations

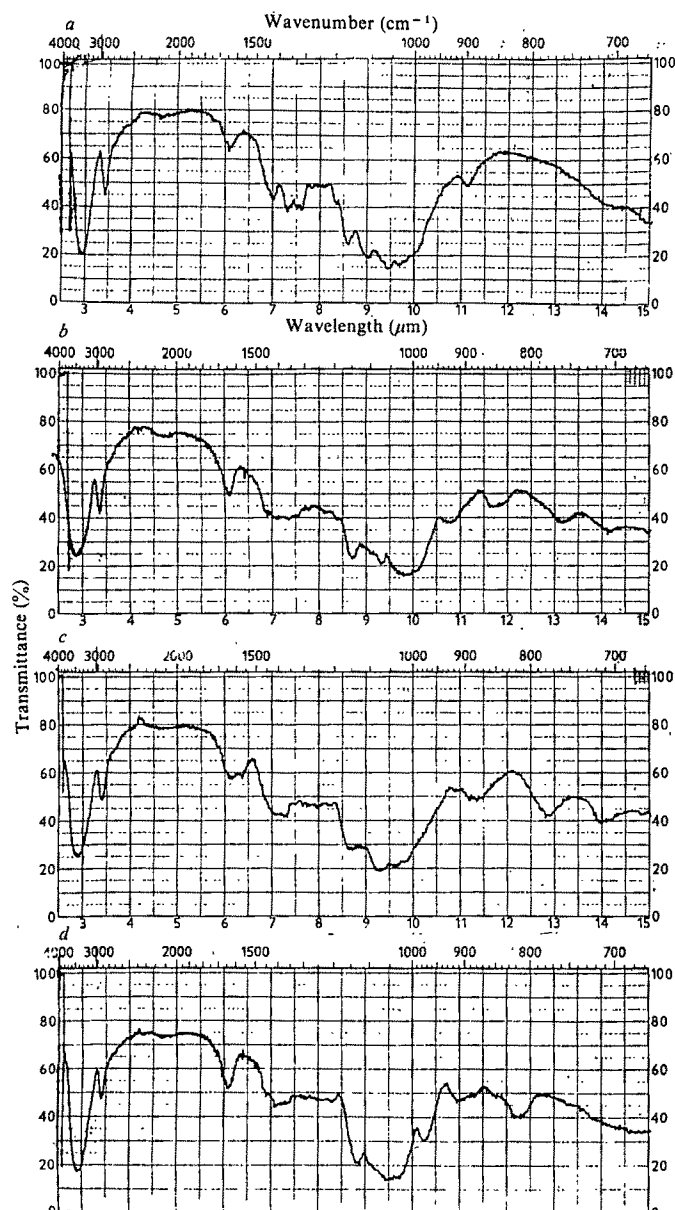


Fig. 1 Transmittance spectra of several polysaccharides dispersed in KBr. *a*, α -cellulose; *b*, amylose; *c*, arabinogalactan; and *d*, mannan. Concentrations are about 1% with respect to KBr. Spectra shown here were obtained using a Perkin-Elmer 137 Infracord Spectrophotometer.

of about 2 mg per 200 mg KBr, and spectra obtained using either a Perkin-Elmer 137 Infracord spectrophotometer or a Perkin-Elmer 257 Infrared spectrophotometer. The spectrophotometer was adjusted to give a 100% transmittance reading at all wavelengths in the absence of the mounted disks. Thus the measured transmittance (%) corresponds to $100 \exp(-\tau_\lambda)$ where τ_λ is the opacity of the laboratory samples. All these spectra (Fig. 1) show the characteristic signatures of polysaccharides, with principal absorptions centred on 3, 3.4, 6 and 9.5 μm . The solid curve in Fig. 2 shows the mean transmittance curve $\exp(-\langle\tau\rangle)$ computed from the spectra in Fig. 1.

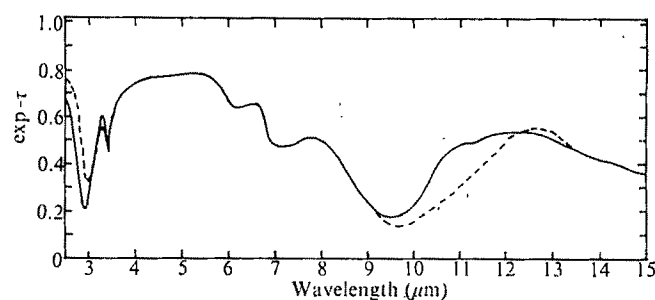


Fig. 2 Mean transmittance spectrum for four samples from Fig. 1. Dashed segments represent optimised variations to give best fit to astronomical spectra.

The agreement of this curve with the spectrum of α -cellulose is close, so our earlier attempts to use a smoothed-out cellulose transmittance curve to represent the properties of interstellar polysaccharides would seem to be justified.

We shall now use our mean polysaccharide transmittance curve to interpret spectra of galactic infrared sources. Using our cascade model for the formation of polysaccharides in mass flows from stars^{2,7} infrared spectra were computed from several sets of transmittance data. Although tolerable fits to astronomical spectra are obtainable with any one of the transmittance curves in Fig. 1 or with the mean curve (solid line) in Fig. 2, the requirement for an excellent fit to a wide range of observations demands minor modifications to our mean polysaccharide transmittance data. These modifications are indicated by the dashed lines in Fig. 2. Figure 3 shows the detailed agreement which is obtained using this modified transmittance data in a source calculation for the BN object. The excellent agreement between observations and theory gives strong empirical support for the modifications proposed here. But even these apparently minor departures from our polysaccharide mean data need interpretation.

The general similarity of the empirically estimated optimal transmittance data to the mean polysaccharide curve is very close except in two respects. (1) The value of $\tau(3\mu)$ for the experimental samples (and the mean curve) are a factor ~1.5 higher than what we infer from astronomical observations. We are most probably seeing here the effect of significant amounts of H_2O associated with freeze-dried terrestrial polysaccharides. Astronomical polysaccharides will be largely free of water, and the 3 μm absorption significantly shallower. A factor ~1.5 could well arise due to this effect. We note that a

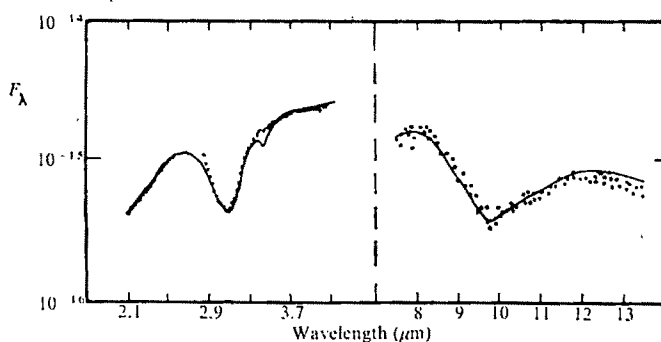


Fig. 3 Solid curve is infrared emission for the source BN calculated for the model of Hoyle *et al.*⁷. The formation temperature of polysaccharides was taken as 850 K and the grain temperature was taken to vary as the inverse square root of the distance from the exciting star. The optical depth of the region of formation of the grains was four times that of the mean sample data of Fig. 2. The points represent the observational data of Gillett and Forrest⁸ and of Merrill *et al.*⁹.

reduction in 3 μm opacity by a factor ~ 1.3 already exists between native cotton cellulose and mercerised cotton, studied by O'Connor³.

Astronomical observations also show minor shifts in the central wavelength of the 3 μm absorption dip with transmittance minima in the range 2.9–3.05 μm compared with minima in the range 2.8–2.9 μm for the laboratory systems we have considered. Wavelength shifts of this magnitude could arise for interstellar polysaccharides due to several effects: variations in the extent of hydrogen bonding between chains, particularly if individual chains are separable; effects of attached side

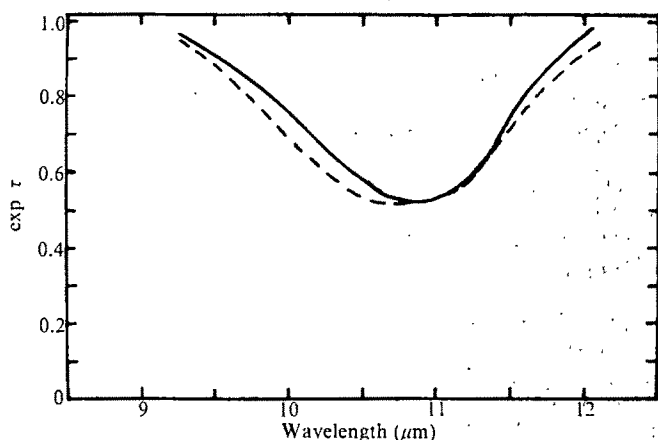


Fig. 4 Required excess opacity (transmittance) in 9–12.5 μm waveband compared with a normalised spectrum of C_3H_6 (propene). The normalisation involves multiplication of the opacities τ in the two cases by factors which equalises the 11 μm transmittance values in the two cases. Observed $\exp -\Delta\tau$ (---); normalised spectrum of C_3H_6 (—).

groups in the polysaccharides; and particle size effects if grains grow to sizes larger than 10^{-5} cm.

(2) The astronomical data require an additional source of opacity in the 9–12.5 μm waveband which must be superimposed upon the polysaccharide transmittance spectrum. This is precisely the waveband where many carbon stars show an excess emission^{4,5} peaking at $\sim 11 \mu\text{m}$ which is readily explained by the presence of a mixture of hydrocarbons. The excess transmittance $\exp(-\alpha \Delta\tau)$ for our dashed curve in Fig. 2 is shown in Fig. 4. The constant α is chosen appropriately for comparison with a suitably normalised laboratory transmittance spectrum⁶ for propene (C_3H_6). The normalisation arbitrarily equalises the value of the transmittance at 11 μm in both cases. The remarkably close agreement leads us to infer the presence of a similar material which is apparently closely associated with interstellar polysaccharides. Hydrocarbons of this type may be produced as an intermediary in the building process of polysaccharide chains in sources, and also as a result of a partial degradation (similar to a coalification process) of polysaccharides in interstellar space.

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Change in nature of stratospheric aerosol collected at 34°S

AEROSOL collected from the stratosphere at Mildura (34.2°S, 142.1°E) has undergone a marked change in character after a period of about seven years with little variation. During the period 1970 to February 1977 aerosol collected by jet impaction at altitudes up to about 28 km has predominantly been sulphuric acid, except for a brief period in January 1972 when particles with a wide variation in morphology were observed¹. The change reported here indicates a substantial incursion of ammonia into the stratosphere, sufficient to almost completely convert the sulphuric acid to one of the ammonium salts, either NH_4HSO_4 or $(\text{NH}_4)_2\text{SO}_4$.

Increased ammoniation is indicated principally by the change in morphology of particles collected (during ascent) on a carbon surface and 'shadowed' with silicon oxide later in the laboratory. The sequence is shown in the T.E.M. photographs in Fig. 1a, b and c. The flights are: a, fl. 667, 2 February 1977; b, fl. 672, 28 April 1977; c, fl. 675, 1 July 1977. The particles shown in Fig. 1 were all collected near 18 km altitude but are in fact representative of the overall aerosol layer between the altitudes of about 16 and 28 km. Particles from flight 667 consist of a small central spherical cap surrounded by rings of acid droplets. This is typical of the aerosol collected over the past seven years and also of aerosols generated from H_2SO_4 in the laboratory and indicates a large proportion of free sulphuric acid in these aerosols. On the April flight, the relative increase in size of the central particles and their 'eroded' appearance points to an increase in ammonia content, while particles collected on the July flight (Fig. 1c) clearly show the morphology of ammonium sulphate with only a very small acid component, that is a flattish eroded dome with a few surrounding droplets. Many of the particles collected at a higher altitude on this flight were found on examination to be in a crystalline form, recognisable as $(\text{NH}_4)_2\text{SO}_4$.

The changed appearance of etch marks left by particles impacted on a copper surface and then subjected to a humid atmosphere is another indication of the changed nature of the aerosol, although this change is non-specific.

Clear etch marks typical of sulphuric acid are evident only on flight 667 (Fig. 1d) whereas the later flights, particularly 672, show a 'splashed' appearance, suggesting a moist or semi-liquid reactive aerosol (Fig. 1e and f).

Chemical testing by thin film techniques with BaCl_2 (refs 2, 3) a specific test for sulphate, shows that for all three flights sulphate is present as a major component in all particles within the radius range of approximately 0.03 to 1 μm (see Fig. 1g, h and i).

Neither the latitude of the NH_3 injection nor the mechanism of its transport into the stratosphere is known. If, however, we use mass loadings calculated from a flight that closely preceded the change a rough estimate of the quantity of NH_3 required to neutralise the H_2SO_4 present can be made. The concentration of particles observed by *in situ* photoelectric counting of individual particles on the flight of 12 November 1976 (J. E. Laby, unpublished) and the size distribution derived from our impaction studies yield a mass of 1.1×10^{-10} kg of H_2SO_4 in a 1 cm^2 column between the tropopause at 12.9 and 30 km. To neutralise this fully to $(\text{NH}_4)_2\text{SO}_4$ requires 3.7×10^{-11} kg of NH_3 , equivalent to a uniform mass mixing ratio of 2.3×10^{-10} . Distributed uniformly over the hemisphere the total mass involved would be a prodigious 10^8 kg. We may, therefore, assume until observations from other latitudes are available that the phenomenon was relatively local. Nevertheless, a very large total mass of ammonia

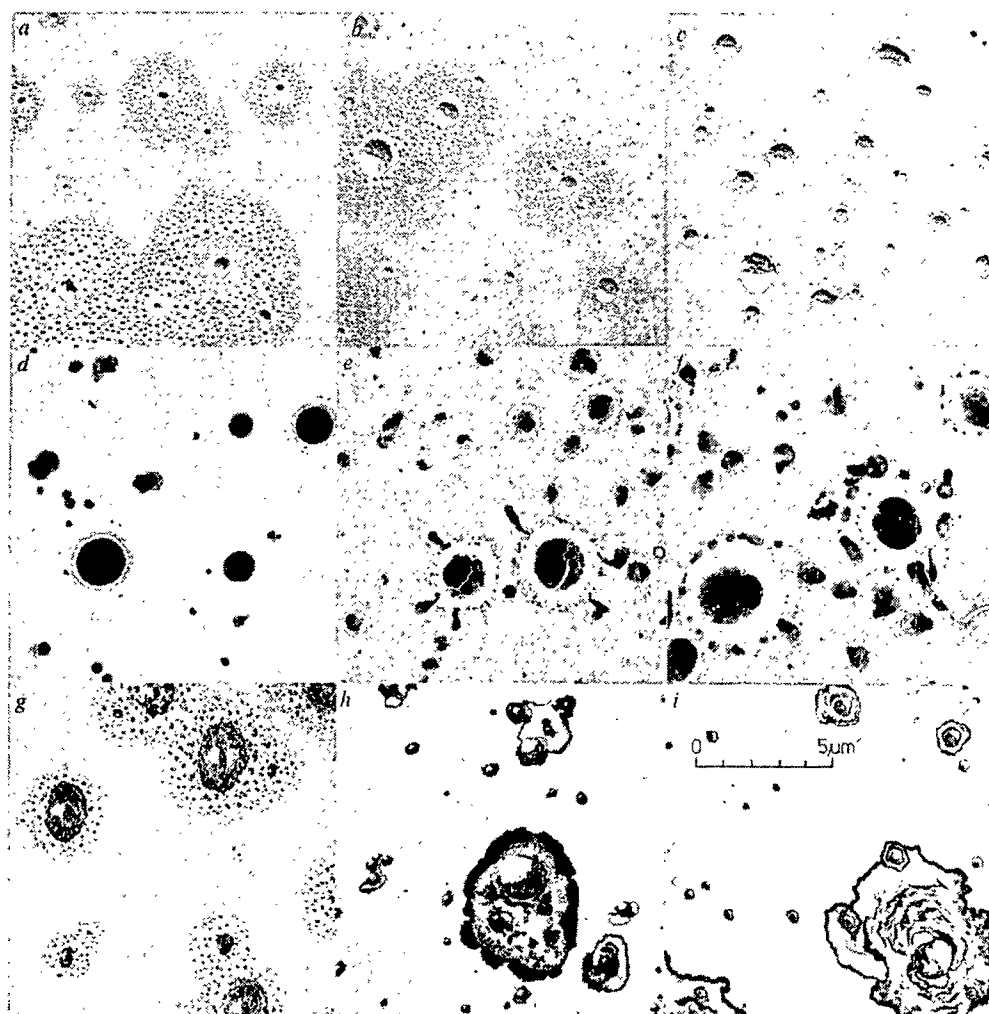


Fig. 1 Particles collected by impactation at approximately 18 km on *a*, 2 February; *b*, 28 April and *c*, 1 July 1977. Surface morphology is shown in the top row which shows particles caught on carbon surfaces and later 'shadowed'. A clear change from H_2SO_4 on the left to $(\text{NH}_4)_2\text{SO}_4$ on the right can be seen. The reactions of particles with a copper collection surface are shown in the centre row and in the bottom row the insoluble BaSO_4 precipitate rings which form after post-coating collected particles with a thin film of BaCl_2 and humidification indicate the presence of sulphate ion.

must have been involved, and it was certainly the most significant injection of the last seven years.

The observations reported here also reveal a phenomenon of particular relevance to stratospheric monitoring: an almost complete change in the nature of the aerosol which simply could not be determined with present remote optical sensing techniques. Direct collection of particles and examination by electron microscopy must remain an essential method of interpretation of stratospheric particulate sources and processes.

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Predicting concentrations in plumes subject to dry deposition

THE source depletion¹ estimation of dry deposition from the atmosphere is less accurate than the physically more correct surface depletion method^{2,3}. Several surface depletion models^{2–9} have been developed but the source depletion method is still used^{1,10}. The errors introduced by using the latter might have important consequences for assessing the impact of deposition from anthropogen sources. Only the recent^{4–6} studies discussed here quantify the discrepancy between the two deposition

methods. We suggest that a ratio R , based on a representative measure of the eddy diffusivity, plume height, and the deposition velocity, should be used to discriminate between cases where the source depletion method introduces significant errors and those where the difference between the two methods is negligible.

Vertical dispersion and dry deposition of material from low sources can be treated in a physically acceptable way by the dispersion equation based on gradient transfer theory (K -theory)¹¹. Gravitational forces are usually neglected and only eddy turbulent transport is considered in the vertical direction. These assumptions are relevant for gases with approximately the same density as ambient air and for submicron particles. Larger particles must be treated separately.

If the dry deposition is discounted, simplified solutions of the diffusion equation lead to the Gaussian dispersion model, which is easy to handle and compare with experimental results, such as tracer studies. Deposition is easily accounted for by reducing the effective source strength as a function of distance. In this 'source depletion model'¹, the vertical concentration profile remains Gaussian and unchanged by the deposition at ground.

An alternative is to solve the dispersion equation with a flux boundary condition at the ground in agreement with gradient transfer theory¹²:

$$\{K(z) \partial c / \partial z\}_{z=z_g} = V_d(z_g) C(z_g)$$

where c is the concentration, z the height, $K(z)$ the eddy diffusivity as function of height, V_d the velocity of deposition and z_g the height of the reference surface. Solution of the dispersion

Table 1 Data extracted from studies of dispersion from a continuous point source 25 m above ground⁴

Distance Parameter	$H(m)$	1 km $K(m^2 s^{-1})$	R	$H(m)$	10 km $K(m^2 s^{-1})$	R	$H(m)$	20 km $K(m^2 s^{-1})$	R
Unstable	200	30.00	15.0	500	50.00	10.0	600	60.00	10.0
Neutral	70	2.50	4.0	190	4.00	2.0	210	4.50	2.0
Stable	25	0.05	0.2	60	0.10	0.2	75	0.15	0.2

H is effective plume height, K is the average diffusivity in the layer, and R is the discrimination ratio.

equation with this requirement results in a 'surface depletion model'^{8,9}, where the vertical concentration profile is modified by the loss of material at the ground. The decreased concentration at the ground in the surface depletion model, verified by experiments^{2,3}, results generally in decreased deposition, compared with the source depletion model.

A recent Gaussian surface depletion model⁵ shows that the source depletion model consistently overpredicts the surface air concentration and the deposition at downwind locations close to the source, and is consequently biased in the opposite direction for locations far from the source. The largest differences appear for low sources in stable stratification over surfaces with relatively large deposition velocities. Some parameters were found to be in error by factors of 3–4 at downwind distances of 10 km (ref. 5).

One-dimensional surface depletion models have been used for long distance travel^{3,6,7}. Considering the amount of airborne material after one day's travel during neutral stratification and with a deposition velocity of 3 cm s^{-1} , the source depletion model underestimates the total amount of airborne material by a factor of 10 compared with this surface depletion model⁶.

Studies with a two-dimensional pseudo spectral surface depletion model⁴ using physically realistic diffusivity and wind profile showed that the latter is important close to the source. Figure 1 shows the suspension ratio, that is the ratio between the vertical integrated airborne mass computed with and without deposition as a function of distance from a continuous point source at a height of 25 m. In the unstable (a) and neutral (b) case, the source and surface depletion models do not deviate significantly. The stable (c) case shows that the surface depletion model yields a lower suspension ratio than the source depletion model at distances $< 6 \text{ km}$ while the opposite is the case at larger distances. Further details on these studies will be published elsewhere⁴.

These results indicate that the deposition close to the source is underestimated and, in general, overestimated at medium distances with a source depletion model. But if a plume is dispersed in stable stratification and, at a distance of 20–30 km from the source, disperses to the ground due to, for example, mechanical turbulence and buoyancy induced by an urban area, the deposition is underestimated by the source depletion model by a factor close to 2. These results might be important for assessment of safety around industrial, such as nuclear, installations.

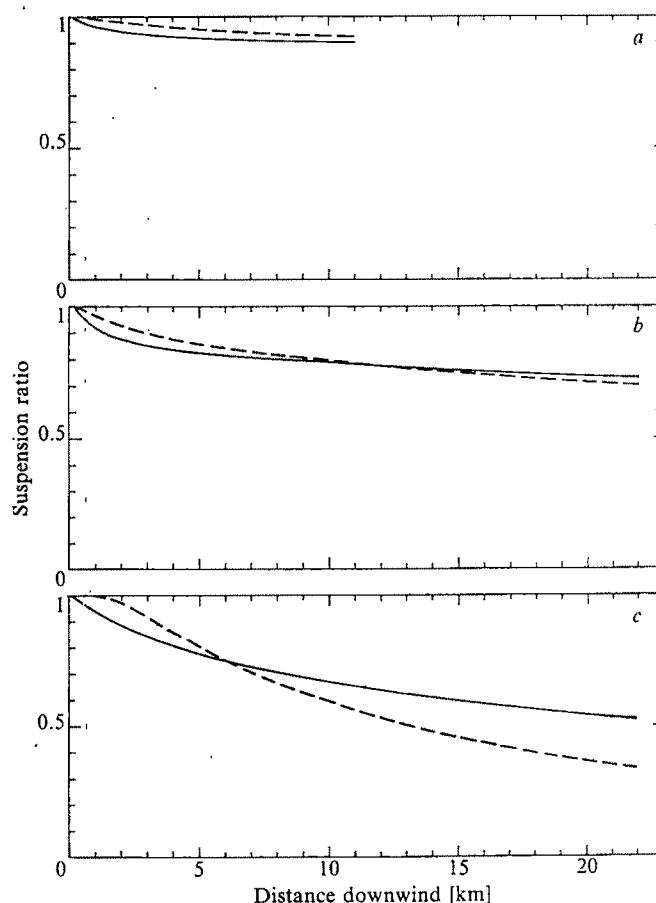
The deposition velocity used here: $V_d = 1 \text{ cm s}^{-1}$ is an average value for SO_2 . Submicron particles, such as sulphate-containing particles, deposit with an approximate velocity of one order of magnitude lower^{13,14}. The diffusivity profiles are relevant for rural country, while the diffusivity, for example, over cold water surfaces, is significantly decreased because of small aerodynamic roughness and cooling at the surface¹⁵. In general, both diffusivity and deposition velocity are functions of surface characteristics and meteorological conditions¹⁴.

The error introduced by the source depletion method is determined by the ratio between the eddy turbulent resistance and the resistance represented by the deposition velocity. A depth L can be determined by the ratio between an effective eddy diffusivity K , and the deposition velocity: $L = K/V_d$, where K is the average diffusivity over the depth H of the atmosphere containing the dispersed material. At large distances from the source, H is equal to the mixing height. At

small distances, $H \approx h + \sigma_z$, where h is the effective source height and σ_z is the standard deviation of the concentration distribution in the vertical direction.

If $L \approx H$, the resistance of the eddy transport is comparable to the deposition resistance, and a source depletion model will not introduce significant errors. This is shown to be the case in neutral and unstable atmospheric conditions with $V_d = 1 \text{ cm s}^{-1}$ (see Fig. 1 and Table 1). During stable atmospheric conditions, L becomes much smaller than H , and a surface depletion model should be used in order to avoid significant errors. A discrimination ratio $R = L/H$ is evaluated on the basis of present studies⁴ (Fig. 1 and Table 1). R can be interpreted as the ratio between a characteristic time for depletion of the depth H of the atmosphere by deposition $\sim H/V_d$, and a characteristic time for diffusion over the same distance $\sim H^2/K$; R is a function of source height and distance. Thus R should not be confounded with the turbulent Sherwood number of vertical surface layer mass transfer. Our studies show that only if $R > 1$, can the source depletion method be applied without significant

Fig. 1 Computed suspension ratio as function of distance from a source at a height of 25 m above ground. Solid line gives the values from a surface depletion model. Broken line gives the values from a source depletion model. Large discrepancies are seen only under the stable atmospheric conditions. a, Unstable; b, neutral; c, stable.



errors, while the surface depletion method can be applied for all values of *R*. This relation has not been previously taken properly into account^{1,10}.
This research is supported by the Danish National Technical SRC.

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Chemical pretreatment
and radial flow of ¹⁴C in tree rings

THE prospect of very large increases in atmospheric CO₂ concentrations by fossil fuel combustion during the next century has prompted an active interest in CO₂ levels of the last century. One way of measuring these concentrations is by doing precise ¹⁴C determinations in wooden tissue of known age. The first question to be answered, then, is whether wood grown during a certain year truly reflects atmospheric ¹⁴C levels of that year. And if whole wood does not, what chemical treatment has to be given to it in order to isolate those substances that do reflect atmospheric ¹⁴C at the time of formation of the ring. Attempts to answer this question have been made but the only conclusion that investigators^{1–5} generally agree on is that all resin fractions are to be distrusted and that the amount of contamination by material from other years of growth depends on tree species. But in some cases resin extraction by organic solvents does not seem to be sufficient^{1,2}. Having installed a new high precision ¹⁴C counting system, to be described elsewhere, we looked at this question again, in view of our further tree ring measurements and the chemical treatment to be applied to our samples. We found that in the tree studied, an oak, radial movement of ¹⁴C across ring boundaries is minor, even where the heartwood–sapwood transition takes place. If year to year

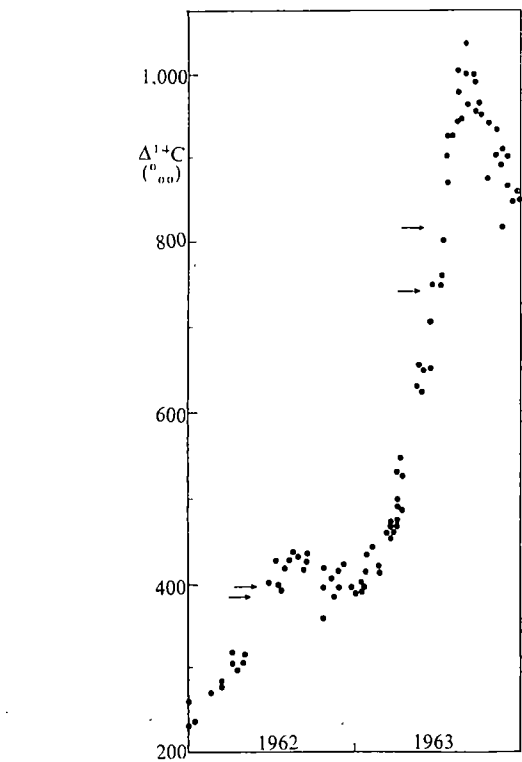


Fig. 1 Δ¹⁴C in atmospheric CO₂ of the northern hemisphere. This figure is a compilation of atmospheric ¹⁴C measurements carried out by Nydal, Vogel and Olsson during 1962–1963. Δ¹⁴C of wood cellulose (arrows) is compared with values from Farmer and Baxter¹, the lower arrows in both years referring to Farmer and Baxter.

variations are small, the effect is negligible. Furthermore, cellulose and wood, treated successively with hydrochloric acid, sodium hydroxide and again hydrochloric acid, both seem acceptable for monitoring past ¹⁴C levels, although they may not exactly refer to the same time of year.
Extensive hydrogen bomb testing raised the ¹⁴C level of the northern hemisphere some 40% from 1962 to 1963. If any ‘radial flow’ of ¹⁴C between successive tree rings exists, the effect is expected to emerge clearly in these year rings. We, therefore, selected wood from these years from the stem of an American oak (*Quercus rubra*), grown in the rural area of the province of Drente, the Netherlands (52° 45’ N, 6° 50’ E) that was felled by a severe storm in November 1972. In this tree the heartwood–sapwood transition took place in the rings of 1961–64.
Four different modes of chemical pretreatment were applied: (1) no pretreatment, that is combustion to CO₂ of whole wood; (2) treatment with 4% HCl at 80 °C during 24 h; (3) treatment with successive 4% HCl (80 °C, 24 h), 4% NaOH (80 °C, 24 h), and 4% HCl (80 °C, several hours), each step separated by thorough washing with demineralised water. This treatment is

Table 1 ¹³C and ¹⁴C determination of *Quercus rubra* after chemical pretreatment

Sample name	Laboratory identification	Pretreatment	Sample size (g)		δ ¹³ C _{PDB} (‰)	Δ ¹⁴ C (‰)
			before pretreatment	after		
AS 1963	GrN-8060	Cellulose	233	96	−23.50	820.6±1.5
AS 1963	GrN-8061	AAA	116	46	−25.24	829.7±1.2
AS 1963	GrN-8101	Acid	130	84	−24.45	809.3±1.7
AS 1963	GrN-8102	Whole wood	72	72	−24.53	805.5±3.5
AS 1962	GrN-8062	Cellulose	278	99	−24.45	389.2±1.5
AS 1962	GrN-8063	AAA	150	59	−25.02	401.3±1.6
AS 1962	GrN-8100	Acid	148	81	−25.41	412.6±1.6
AS 1962	GrN-8099	Whole wood	91	91	−26.03	409.4±1.6

routinely applied to most organic samples in our laboratory (AAA treatment); (4) preparation of α -cellulose, successively obtained by extraction with a 2:1 mixture of benzene-ethanol during several hours, rinsing with ethanol, bleaching by a NaClO_2 solution and acetic acid⁸, followed by treatment with 4% NaOH (80 °C, 24 h) and 4% HCl (80 °C, several hours). The last three steps again are separated by washing with demineralised water.

Before using methods (2), (3) or (4) the wood has to be chipped to matchstick size. The size of the final samples as they are measured is 50–55 l of CO_2 gas at NTP. The samples were kept under high pressure in small stainless steel containers. Therefore, special care had to be taken that the tiny amount of gas needed for ^{13}C determination had not undergone isotope fractionation with respect to the bulk of the sample. The results are given in Table 1 and plotted in Fig. 2, together with the results of Farmer and Baxter¹. The $\Delta^{14}\text{C}$ values are obtained by

$$\Delta^{14}\text{C} = ((A_s - 0.95 A_{ox}) / 0.95 A_{ox}) \times 1,000$$

where A_s and A_{ox} are the age and fractionation corrected ^{14}C activities of the sample and oxalic acid (samples to $\delta^{13}\text{C} = -25.0\text{‰}$, oxalic acid to -19.0‰).

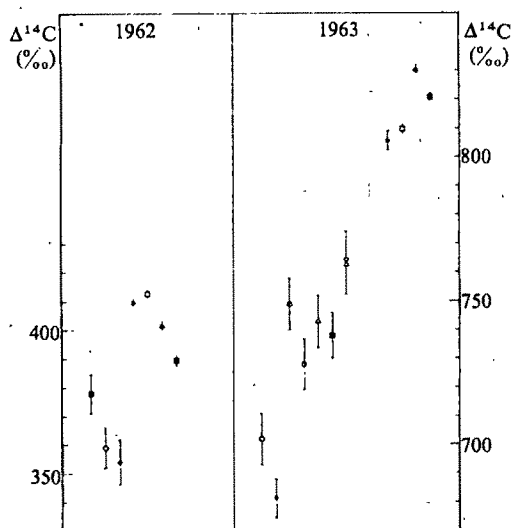


Fig. 2 $\Delta^{14}\text{C}$ in tree rings after different chemical pretreatments. The small error bars refer to this paper the larger to Farmer and Baxter¹: ● Whole wood; ■ cellulose; □ acid; ▲ AAA; ○ acetone; ● acetone-petrolether; × lignin; △ acid-alkali; ● acetone-acid-alkali.

The $\Delta^{14}\text{C}$ of whole wood from 1962 is 20‰ higher, that from 1963 15‰ lower than the respective cellulose fractions. With regard to a difference between the atmospheric ^{14}C levels in these years of about 400‰ this points to a 4–5% 'mixing' of organic material, presumably resins, across the 1962–63 boundary. Compared to the AAA pretreatment these figures are +10‰ and –25‰ respectively. Treatment with acid does not change this picture much. The AAA sequence, however, gives a result which is about 10‰ higher than cellulose both in 1962 and 1963 and when another, arbitrary ring was tried:

$$\begin{aligned} 1971 \text{ cellulose } \Delta^{14}\text{C} &= +496.4 \pm 2.8\text{‰} \quad \Delta^{13}\text{C} = -23.88\text{‰} \\ 1971 \text{ AAA } \Delta^{14}\text{C} &= +512.5 \pm 3.6\text{‰} \quad \Delta^{13}\text{C} = -25.27\text{‰} \end{aligned}$$

An explanation for this could be that the AAA treatment attacks earlywood more than it does latewood. The stratospheric leak occurring each year at our latitudes causes ^{14}C levels to be much higher in August than in early spring, at least several per cent during the 1960s. An alternative explanation is that the AAA treatment does not remove lignin or its fragments. Lignin is added to the cell structure in later stages of cell growth⁷. Full lignification of cells in *Pinus ponderosa*, for instance, occurs about a month later than cellulose formation. This hypo-

thesis is supported by the $\delta^{13}\text{C}$ values found, as lignin generally is more depleted in ^{13}C than cellulose. Also Wilson and Grinsted⁸, in searching for climatic information from stable isotopes in very thick tree rings, have found a displacement in time of the lignin curve against the cellulose curve.

Furthermore, Fig. 2 shows that Farmer and Baxter's $\Delta^{14}\text{C}$ values are consistently lower than ours. Cellulose for instance is $12 \pm 7\text{‰}$ lower in 1962 and $83 \pm 8\text{‰}$ lower in 1963. This cannot result from a latitudinal effect, as Nydal has shown it to be virtually non-existent⁹. Moreover, their tree has grown at almost the same latitude (Glengarry, Scotland, 57° N) as ours. Both trees grew in rural environments. In our view, the observed effect follows either from a shift in time of major growth, that is, the Glengarry tree grew earlier during the year than the Drente tree, or local recycling of CO_2 is involved, that is, the incorporation in the ring of CO_2 from decaying organic matter in the forest. The rate of increase of atmospheric ^{14}C levels was 40‰ per month and 90‰ per month until August in 1962 and 1963 respectively (Fig. 1). This observation supports the first hypothesis but does not exclude the second. The second hypothesis is supported by the fact that the Drente tree was planted in 1880 and did not grow in a forest, while the Glengarry tree was only planted in 1937 in a forest.

This project is supported by the Netherlands Organization for the Advancement of Pure Research (Z.W.O.).

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Received 19 October; accepted 15 November 1977.

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Unusual room temperature afterglow in some crystalline organic compounds

STRONG room temperature phosphorescence studies of certain doped solid systems^{1–4} have shown that the characteristic phosphorescence peak wavelengths agree with those established for the corresponding dopants dissolved in 77K systems; the room temperature lifetimes being smaller by a factor of 1.5–2, depending upon the nature of the matrix. We have recently observed an unusual new phenomenon—a weak afterglow from certain of the dopant compounds including carbazole, dibenzothiophene, dibenzofuran and triphenylene when examined as pure crystalline solids. This weak afterglow has different peak wavelengths and lifetimes, but in dilute solid solution form in room temperature resin systems there is no trace of the weak afterglow properties. Melting and resolidification of these pure aromatic compounds do not affect the weak afterglow, and, after vacuum sublimation of carbazole, the afterglow is still observed from the purified product. We believe that we can rule out an impurity effect and this view is reinforced as the afterglow intensifies after recrystallisation from ethanol. As the crystals probably improve on recrystallising, the crystal quality may play a role in this phenomenon.

The phosphorescence spectrum of a dilute (1%) solid solution of carbazole in a thermoset resin at room temperature is shown in Fig. 1b (recorded using an EMI

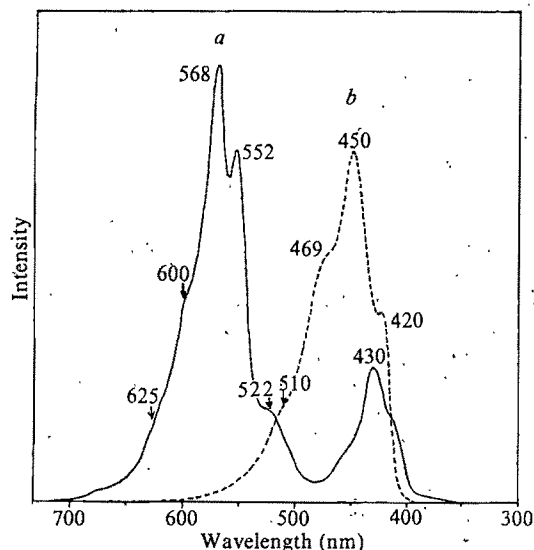


Fig. 1 *a*, Room temperature afterglow spectrum of crystalline carbazole. *b*, Phosphorescence spectrum of 10^{-2} Molal solid solution of carbazole in a thermoset resin at a gain factor of 0.05 relative to *a* at the same setting of the spectrophosphorimeter.

photomultiplier type 6256). The main peaks at 420, 450 and 469 nm correspond well with the phosphorescence emission peaks in *n*-heptane at 77K (ref. 5). The room temperature lifetimes of the 420 and 450 nm peaks are 3.8 and 4.85 s respectively, which is a reduction by a factor of 2 compared with the 77K system. The weak afterglow spectrum is also shown in Fig. 1*a*, at a gain factor of 20 for 100% solid crystalline carbazole at an identical setting of the spectrophosphorimeter. This spectrum shows peaks at approximately 430 nm and also at 522, 552 and 568 nm wavelengths. The 430 nm peak has a decay time which is faster than the chart recorder response, 0.3 s. The other peaks at 552 and 568 nm show mean lifetimes of 0.7 and 0.88 s respectively. Figure 2*b* shows log intensity, plotted against time for the strong 450 nm room temperature phosphorescence of the carbazole solid solution in resin and Fig. 2*a* shows the corresponding curve for the weak afterglow peak at 552 nm. As the plot for the weak afterglow is a straight line, it is possible that the weak afterglow, having a first-order decay, has origins in a metastable state, as does the normal 450 nm triplet.

Fig. 2 Plots of $\ln I$ against time. Plot *a* for 552 nm peak of afterglow (see Fig. 1 *a*); *b* for 450 nm phosphorescence peak (see Fig. 1 *b*).

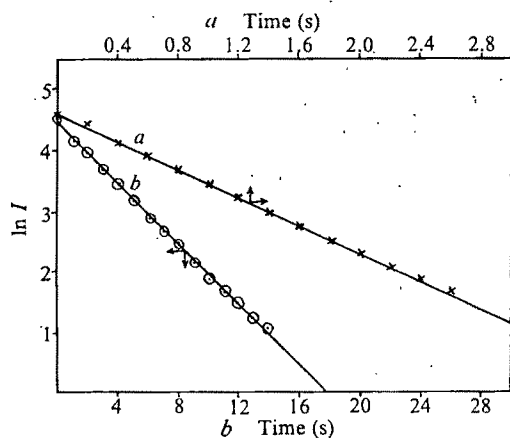


Figure 3*a* shows the total emission spectrum of crystalline carbazole, which is mainly fluorescence, with a negligible phosphorescence above 500 nm. The totally fluorescent emission spectrum of a 10^{-2} Molal solid solution of carbazole in PMMA is shown in Fig. 3*b* (both curves were recorded as observed with an EMI photomultiplier type 9524B). Figure 3*a* for crystalline carbazole, was recorded at half the slit width used for Fig. 3*b*, giving the latter curve a gain factor of times 2. The excitation for both spectra was achieved by the 312 nm mercury lamp line through a grating monochromator. Comparing the curves, Fig. 3*b* for the solid solution shows emission typical of molecular carbazole in dilute solutions in solvents such as ethanol and cyclohexane, apart from some self-absorption at the shoulder at 343 nm (ref. 6). Figure 3*a* for crystalline carbazole, however, has a much broader peak which is red-shifted by 67 nm compared to the higher peak of curve *b* and, in addition, curve *a* does not show any well defined vibrational structure in contrast to the other three emission curves shown in Fig. 1 and Fig. 3*b*. We note that although the fluorescence peak intensity at 423 nm in Fig. 3*a* is about 2 or 3 orders stronger as compared with the afterglow peak intensity at 430 nm in Fig. 1*a*, the peak shapes and position are very similar in both cases. This result indicates that the 430 nm peak in the weak afterglow spectrum has origins from emissive states electronically similar to those yielding fluorescence in crystalline carbazole.

The characteristic strong room temperature phosphorescence properties of carbazole in the thermoset resin (Fig. 1*b*) shows that this is radiative emission from the triplet state. But from the magnitude of the peak intensity of the room temperature phosphorescence, we suspect that the triplet state may be populated by a donor species. The crystalline form of carbazole, however, shows no blue phosphorescence, indicating that either insufficient numbers of normal triplet states are populated, or that the energy is channelled to lower emissive states responsible for the peaks at 522, 552 and 568 nm (see Fig. 1*a*).

Reappraising the afterglow (curve *a*) and phosphorescence (curve *b*) spectra in Fig. 1, we note that the vibrational spacings of the peaks are similar in both cases. The overall shapes also match each other, apart from a magnitude inversion of the 552 and 568 nm peaks compared to the 450 and 469 nm peaks.

Wavelengths of various peaks and shoulders are listed in Table 1*a*. There is good correlation between the two columns, all peaks in column 1 being red-shifted by approximately 90–100 nm. The peaks at 522, 552 and 568 nm show first-order decay rates of afterglow intensity after the illumination is stopped; however, the lifetime is of an order of magnitude smaller compared to the 450 nm phosphorescence peak (≈ 0.7 s, as opposed to ≈ 4.5 s). These results indicate that the afterglow is due to an emission to S_0 of the monomer or to a vibronically similar ground state. Furthermore, T'_1 (metastable state giving weak afterglow) lies below T_1 , the monomer triplet state (the difference being $\approx 4,106 \text{ cm}^{-1}$). Comparisons of the various peaks and shoulders of fluorescence emission spectra of crystalline carbazole and a 10^{-2} Molal solid solution of the same compound in PMMA are shown in Table 1*b*. Apart from a correlation of the two weak shoulders on the long wavelength tail of the emission, there are great discrepancies in the two spectra. This indicates that the excited state in crystalline carbazole (probably a first excited singlet, S'_1) has facilities for vibronic energy dissipation and, furthermore, the strong emission at 423 nm is unique to the crystalline samples. An explanation for these results could be the presence of dimers in the crystalline carbazole, or the formation of an excimer during photoexcitation. Excimer emission from liquid solution is well known for pyrene⁷

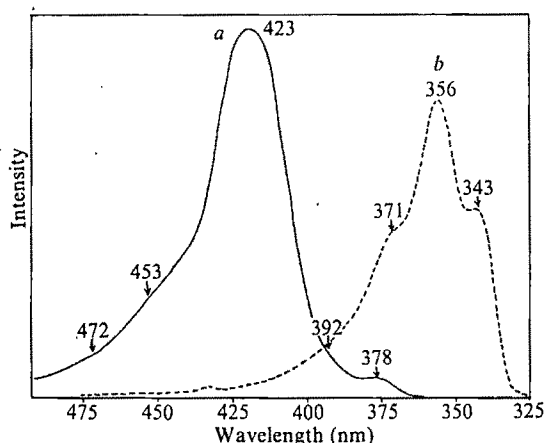


Fig. 3 Fluorescent emission spectra of carbazole. *a*, Crystalline carbazole. *b*, 10^{-2} Molal solution in a thermoset resin at a gain factor of 2 compared to *a*. Excitation for both spectra provided by 312 nm line through a grating monochromator from a high-pressure mercury lamp.

which shows a broader and red-shifted spectrum compared to the pyrene monomer emission. We think that random variations of orientation and spatial variations which may be responsible for such broadening in the liquid state would be inhibited in solid crystalline carbazole. The relatively sharp peaks compared to those for liquid state dimers⁷ in the fluorescence and weak afterglow spectra of crystalline carbazole are thus not altogether surprising.

Our results show, therefore, that the monomer in solid solution exhibits normal emission, corresponding to the first excited singlet state and the first excited triplet state. In the crystalline state of pure carbazole, the corresponding emissions of fluorescence and of weak afterglow are completely different, the fluorescent spectrum being broad and showing little or no vibrational structure, indicating energy dissipation from this excited state. The afterglow spectrum shows the unmistakable vibrational bands of transitions between two relatively stable states. We propose that the weak afterglow emission originates from the triplet state of a dimer or carbazole pair. The fluorescence of the crystalline carbazole would be expected to originate in the excited singlet state of the same species, however, the spectral broadening suggests some excimeric character, implying a less stable pairing in the excited singlet as compared with either a dimer triplet state or a dimer ground state.

Table 1 Comparison of afterglow and fluorescence emissions

<i>a</i> Afterglow or phosphorescence emission	
Crystalline afterglow possibly dimer or excimer $\lambda(T_1)$	Dilute glass phosphorescence monomer $\lambda(T_1)$
522	420
552	450
568	469
600	510
625	—
<i>b</i> Fluorescence emission	
Crystalline possibly dimer or excimer $\lambda(S_1)$	Dilute glass monomer $\lambda(S_1)$
379	—
392	—
423	—
—	343
453	356
473	371

We thank ILEA for the award of a research assistantship to N.H.

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Received 30 September; accepted 14 November 1977.

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Utilisation of soapstone in Labrador by Indians, Eskimos and Norse

MUCH of our knowledge of prehistoric peoples of North America comes from the study of their utilisation of imperishable lithic (geological) resources. In some cases geochemical differences in the origin of a particular lithic material have resulted in trace element differences which can be used to characterise the geological source of this material¹. Both geochemical and archaeological studies indicate that the rare earth element (lanthanide) distributions in soapstone (steatite) can be used to characterise this commonly used lithic material^{2–4}. The rare earth elements (REE) in artefacts from Labrador and Newfoundland, measured by instrumental neutron activation analysis and reported here, suggest that the same soapstone sources have been used by Indians, Eskimos, and Norse at various times over the past 4,000 yr.

Apart from the importance of cultural history in general, Labrador, because of its location at the juncture of the arctic and subarctic zones, has an intriguing prehistory. The environmental and cultural boundaries in this region have been studied as they shifted through time^{5–7}. The Indians and Eskimos who occupied this area at various times utilised soapstone in characteristic ways. For instance, the Indians (Montagnais-Naskapi) who were present in southern Labrador 7,500 yr ago made soapstone plummets (fishing sinkers?) which have been found at Late Maritime Archaic (4,000–3,500 yr BP) sites in central and southern Labrador as well as Newfoundland. Dorset Eskimos (who first appeared in central Labrador from the north about 4,000 yr ago) used soapstone for oil lamps, cooking pots and several types of amulets. These artefacts are found in various Dorset sites from Newfoundland north which range in age from 2,500–1,000 yr BP. Later, in Labrador Eskimo times (500–150 yr BP) soapstone was extensively used for posts and lamps until replaced by European technology. A soapstone spindle whorl, found at L'anse aux Meadows, near the northern tip of Newfoundland, is part of the evidence for this early European settlement in North America^{8–9}. Similar artefacts are common in Norse sites in Iceland and northern Europe.

Soapstone, having discrete and localised sources, could not have been available to all of these groups in one locality. Consequently, trade or travel to secure artefacts or raw materials must have been common within and between cultural boundaries. The small size of most soapstone quarries and the lack of information about quarries during the past century in Labrador makes many of the source locations difficult to find. Nevertheless, matching artefacts from the same, but unknown, quarry does provide valuable information about resource procurement. As each geological deposit of soapstone may have formed under different conditions of contact or regional metamorphism from a variety of older rock types, trace element

concentrations vary considerably between different areas. Having analysed over 50 sources of soapstone in eastern North America, we have shown that each formation can be characterised by its trace element contents^{3,10}. In particular, the rare earth elements ($Z = 57-71$) vary in both absolute and relative abundances. The differences can be shown graphically by plotting the normalised concentration of the particular rare earth element (obtained by dividing its concentration in the soapstone by that found in chondritic meteorites) as a function of atomic number³. The curves (REE patterns) reflect the slightly different geochemical behaviour of these elements which result from differences in ionic radii and in the case of Eu, the ionic charge.

Each geological formation may have rocks which vary considerably in their mineralogies, but if they contain sufficient quantities of talcose minerals (such as talc, chlorite, antigorite, and tremolite), they have the physical characteristics which would have allowed them to be utilised. While we have observed that some of the trace elements (for example, Fe and Cr) do depend upon the mineralogy, it seems that the REE do not. Figure 1 shows three examples of the variations observed in REE patterns for soapstone from a particular geological region. Based upon the analysis of between 5 and 20 samples from these and several other individual geological formations, we conclude that the REE patterns are essentially the same in shape (are parallel) but differ in absolute magnitude. For REE concentrations which are about an order of magnitude greater than the chondritic concentrations, the magnitude of the differences observed between samples is about a factor of 2. When the REE concentrations in a soapstone formation are the same as or lower than the chondritic concentrations, the differences between samples from the same source are larger, differing by as much as a factor of 5, but the patterns are approximately parallel. This gives some insight into how well an artefact might be expected to match a sample from a quarry in order to establish the quarry as the source region.

Matching the REE patterns in soapstone artefacts to the source of the raw material is based on the assumption that the quarry can be located and sampled. Although over 50 different soapstone source regions (varying considerably in size) have been differentiated on the basis of their REE patterns, the analysis of artefacts from eastern North America suggest that others have not been found. This has been a particularly difficult problem because the analysis of over 60 Eskimo and Indian artefacts grouped together, according to their REE patterns, suggest that at least eight soapstone sources were used over the past 4,000 yr. But only four of these have been located. Two quarry areas have been sampled in Labrador, one at Freestone Harbour in the Davis Inlet area south of Nain and the other at Moore's Island near Okak, north of Nain. On the island of Newfoundland the large outcrops at Fleur-de-Lys were sampled, as well as an outcrop a mile from the L'anse aux Meadows site (provided by the Conservation Division of Parks, Canada).

The L'anse aux Meadows quarry is of particular interest as it may have been the source of the Norse spindle whorl (Fig. 2). Other use of the L'anse aux Meadows quarry is indicated by a plummet, from the 4,000 yr old Maritime Archaic Indian site at Rattlers Bight in the Hamilton Inlet region, which also has a matching REE pattern¹¹. This relatively distant source was not the most common one for the Indians in this region as most of the plummets from this site have the same REE patterns, but the pattern does not match any of the quarries sampled. One might assume a more local source. In another Maritime Archaic site further north at Windy Tickle (Davis Inlet) the only two plummets have REE patterns which match a nearby soapstone outcrop at Freestone Harbour.

By 2,600 yr BP the Dorset Eskimo culture had advanced into central and southern Labrador and by 2,000 yr BP, into Newfoundland. Dorset people used some of the same quarries or quarry locations as the earlier Maritime Archaic Indians. The Freestone Harbour soapstone was used extensively in the Nain

and Okak areas between 2,700–1,400 yr BP. Soapstone from the Okak region (Moore's Island) was another relatively local source used extensively by the Dorset Eskimos in the Nain area based upon the matching of REE patterns in bowls to those of the quarry (Fig. 2). There was also the rare utilisation (10% of the samples) of the soapstone from the two quarries in Newfoundland. The problem still remains that nearly half of the 30 Dorset artefacts from central Labrador do not match any of these four quarries.

The method of transmission of information about soapstone quarries within the different Indian and Eskimo cultural

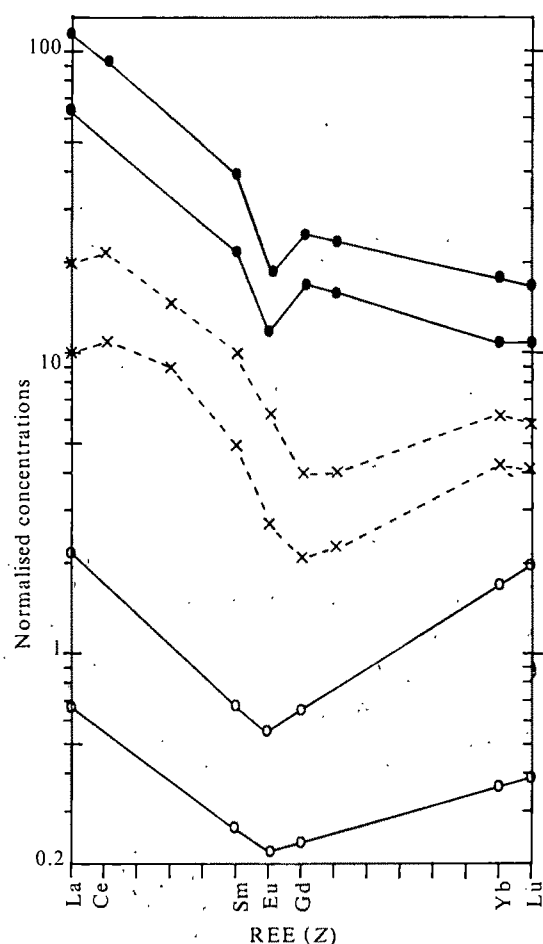


Fig. 1 Chondrite normalised rare earth element (REE) distribution patterns of some soapstone outcrops in Virginia and Crete. The Virginia samples are from the Albemarle–Nelson County region and the two lines (X) give the extremes of the range for 15 different samples analysed. The two lines (●) define the range of 14 samples from a relatively large region in Crete which is assumed to be a single large soapstone formation. The patterns (○) noted as Crete B define the range observed for 13 samples from the Gonia region of Crete. In all cases the other samples are approximately parallel to the curves shown but differ somewhat in magnitude within the range defined by these curves. This gives an indication of what type of variations might be expected for artefacts from a particular quarry.

sequences or between ethnic groups at times of culture area changes is not known. Oral tradition must have been important during periods of continuity, but prehistoric peoples (and Norse as well) must have been keen observers of geology. Prehistoric people of Labrador used many different lithic materials, most of whose source locations are unknown today. It is unfortunate that Western culture replaced native traditions so rapidly and completely before these observations could be recorded. We are now confronted with the difficult task of rediscovering the sources of many culturally important lithic materials for use in studying cultural movement and contact. The use of REE patterns to match artefacts to the geological source of the

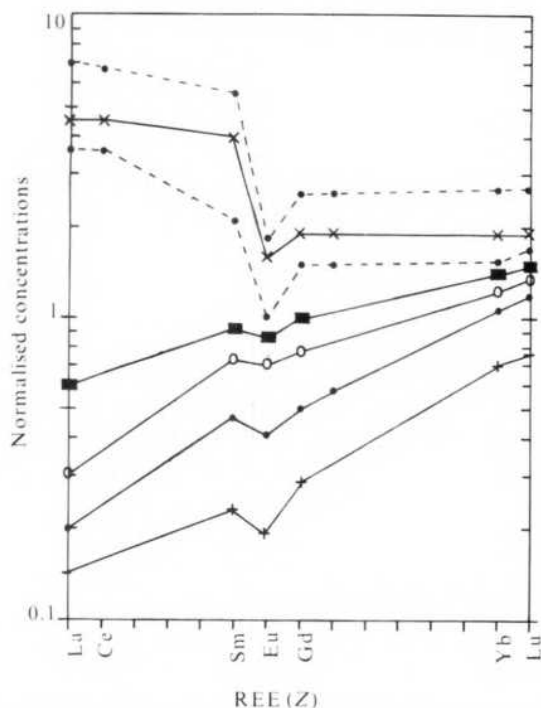


Fig. 2 Chondrite normalised REE patterns for two quarries and four artefacts interpreted as originating in these quarries. Quarry L (●) is a sample from a soapstone outcrop in northern Newfoundland near L'Anse aux Meadows and the Norse artefact (■) is the spindle whorl from the L'Anse aux Meadows site. The Indian artefact (○) is a plummet from a Maritime Archaic site at Rattler's Bight in Labrador while the Eskimo artefact (+) is from a rectangular cooking vessel from a Dorset Eskimo site in the Nain region further north. The dashed curves (●) outline the range of three samples of soapstone from outcrops in the Okak region (north of Nain) and the Eskimo artifact (×) is another cooking vessel from the same Dorset site in the Nain region.

soapstone provides an important new tool for the study of lithic resource procurement. Most of the soapstone tested from prehistoric sites to date seems to be from local quarries. The presence of occasional exotic materials argues for the existence of exchange networks maintained by social rather than economic need.

This research was supported by NSF grant 41500. Eskimo and Indian artefacts from the Smithsonian Institution were collected with support from The National Geographic Society 1971–72, and the Smithsonian Research Foundation, 1973–76. L'Anse aux Meadows spindle whorl and quarry samples were obtained through the cooperation of J. C. McCawley, Conservator for Parks, Canada. Freestone Harbour samples were obtained through the courtesy of Saunders of Goose Bay, Labrador, and Moores Island samples from S. Cox and N. Ettlinger. Crete samples were provided by M. Becker.

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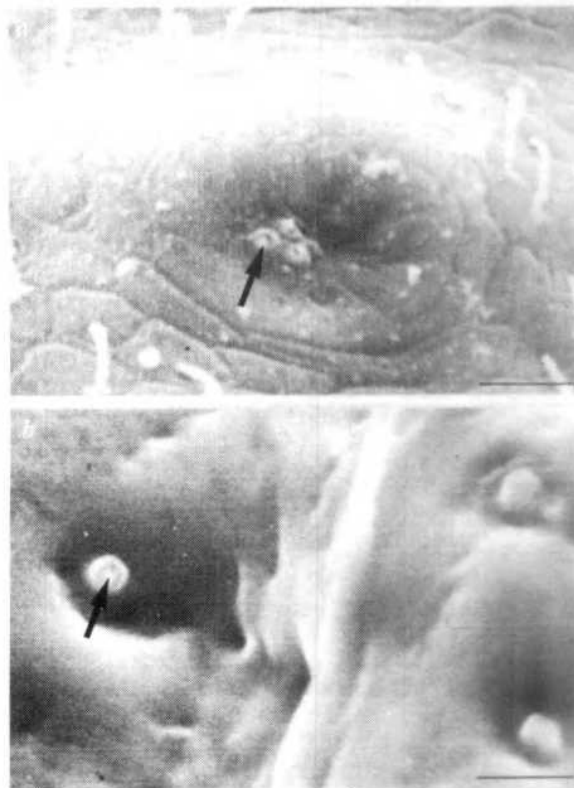
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Inhibition of oesophageal peristalsis in the lobster after chemical stimulation

PREVENTION of hyperphagia by some mechanism is essential to feeding animals. To date relatively few such control mechanisms have been studied in detail. Termination of feeding by internal inhibitory feedback from stretch receptors is documented for *Phormia regina*¹, *Locusta migratoria*², *Chortoicetes terminifera*³ and *Aplysia californica*⁴. Chemosensory adaptation has been suggested for *Phormia*¹ and *Chortoicetes*³, and in *Locusta* activation of foregut stretch receptors will, through a nervous and hormonal pathway, close the terminal pores of chemosensory sensilla on the maxillary palps⁵. We present here evidence showing that stimulation of a pair of oesophageal contact chemoreceptor organs can slow the ingestion rate and terminate feeding in *Homarus gammarus*.

The anterior oesophageal sensors (AOS) were first described in *Homarus* by Allen⁶ and were described in more detail in the crayfish *Astacus leptodactylus* by Orlov⁷ (for a review of the sensory innervation of the foregut of decapod crustaceans see ref. 8). These sensors are bilaterally symmetrical and their cuticular endings are situated in the clefts between the anterior and lateral lobes of the oesophageal/cardiac sac valve (data not shown). Each anterior oeso-

Fig. 1 Scanning electron micrographs of the cuticular modifications associated with the anterior oesophageal sensor. *a*, Grouped endings located in depressions in raised hillocks on the lateral wall of the anterior lobe of the oesophageal/cardiac sac valve. *b*, Single endings present deep in the cleft between the anterior and lateral lobes of the valve. It is possible that the sensilla have terminal pores (arrowed). The single endings bear a marked resemblance to those described for contact chemoreceptors in insects^{9,10}. Scale bar, 10 µm in *a*, 4 µm in *b*.



phageal sensor is innervated by a dorsal branch of the ipsilateral superior oesophageal nerve (SON) and can be divided readily into two populations of receptor neurones; 1, 50–60 bundles of 3–5 small (15–20 μm long axis) uniterminal bipolar neurones; and 2, 250–300 similar small neurones which are present singly. The cuticular modifications associated with the dendrites can be divided similarly (Fig. 1). In addition there are between two and five large (60–80 μm long axis) bipolar neurones whose axons also travel in the anterior oesophageal sensor nerve and whose long dendrites travel over the surface of the cardiac sac and oesophagus in the region of the valve.

Recording and stimulation of selected nerves was carried out with silver wire hook electrodes using conventional techniques. In all experiments the bathing medium was clean, cool seawater. The organ was chemically stimulated with an extract of *Mytilus edulis*. The gills and mantle of a fresh mussel were homogenised in approximately the same volume of seawater. This was applied directly on to the anterior oesophageal sensor with a glass pipette inserted through a hole cut in the cardiac sac. The afferent axons of other chemoreceptors in the area were cut.

The oesophagus undergoes peristalsis for some time after initial dissection, and recordings from the superior oesophageal nerve during this time reveal a complex rhythmical burst which can be shown to occur during oesophageal dilatation. This burst was used as an indicator of oesophageal dilatation during peristalsis. The frequency of bursting is relatively constant at any one time but varies between 0.1 Hz and 0.33 Hz. Suprathreshold electrical stimulation of the anterior oesophageal sensor nerve with a train of rectangular pulses of width 0.6 ms and with a pulse interval of 500 ms has two effects (Fig. 2). In this preparation the burst frequency dropped from its original level (about 0.28 Hz) to approximately 0.1 Hz; this was accompanied by a drop in the number of spikes in each burst. On cessation of stimulation the burst frequency and the number of spikes per burst increased but did not reach their original level. Application of *Mytilus* extract directly on to the anterior oesophageal sensor mimics the effect of electrical stimulation of the sensory axons by reducing the burst frequency (Fig. 3), although there is no reduction in the number of spikes per burst. Continued application can terminate bursting, and to be effective the extract must be closely and continuously applied.

The anterior oesophageal sensors are classified as contact chemoreceptors on morphological grounds and direct ex-

Fig. 2 Effect of electrical stimulation of the anterior oesophageal sensor nerve (black bar) on rhythmical bursting in the SON. The number of spikes in each burst (a) and the burst frequency (b) are plotted against the time of occurrence of each burst. Burst frequency is calculated as the reciprocal of burst interval which is measured from the start of one burst to the start of the succeeding burst.

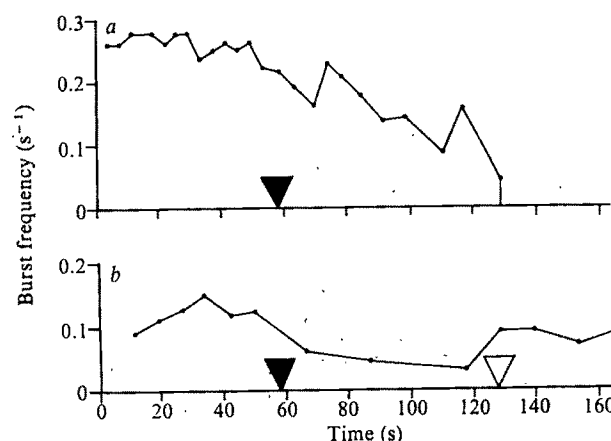
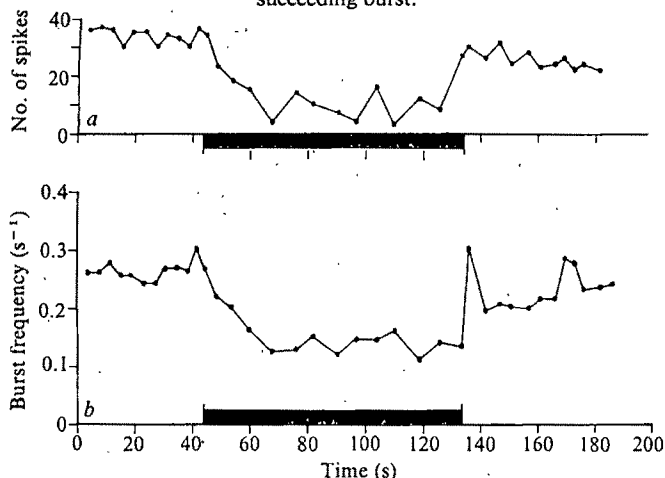


Fig. 3 Effect of chemical stimulation of the anterior oesophageal sensor on rhythmical bursting in the superior oesophageal nerve. (a) and (b) are different experiments. Application of *Mytilus* extract (solid arrow) slows and stops bursting (a). In (b) burst frequency increases on removal of the pipette (open arrow) but without washing the organ.

perimental evidence is not yet available. The positions of the receptor organs are such that they become available for stimulation only when the cardiac sac is filled to capacity and the oesophageal/cardiac sac valve is stretched open. The solitary endings lie deep in the cleft between the lobes of the valve while the grouped endings spread on to the lateral walls of the anterior lobe. It is unlikely that these positional differences reflect functional differences, the more complex arrangement of the grouped endings probably serving to prevent causal stimulation during feeding. The observed difference between electrical and chemical stimulation can be explained by the presence in the anterior oesophageal sensor nerve of the axons of the small group of large bipolar neurones. These are presumptive mechanoreceptors that may respond to stretch in the region of the oesophageal/cardiac sac valve and could also be involved in the control of the peristaltic rhythm.

These observations suggest an alternative control mechanism for feeding processes, dependent upon chemical rather than mechanical feedback to higher centres. It is possible that such methods of controlling food ingestion will be found more commonly, especially amongst decapod crustaceans.

R.M.R. was supported by an SRC research studentship.

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Received 8 September; accepted 10 November 1977.

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A *Rhizobium* mutant incapable of nodulation and normal polysaccharide secretion

THE surface carbohydrates of *Rhizobium*, including both the lipopolysaccharide and the extracellular polysaccharide, are thought to participate in the infection and nodulation of legumes by these Gram-negative bacteria^{1–3}. We report here the isolation of a mutant of *Rhizobium leguminosarum* which produces diminished amounts of extracellular polysaccharide

(that is, the carbohydrate material present in cell-free filtrates of bacterial cultures) and which fails to nodulate pea, its normal host.

Two antibiotic resistance markers were introduced, by spontaneous mutation, into *R. leguminosarum* strain 128c53 (obtained from J. C. Burton of Nitragin Co.). Approximately 10^{10} cells of this strain growing in minimal liquid medium⁴ were concentrated by centrifugation. The cell pellet was resuspended in 0.1 ml of medium and spread on a minimal agar plate⁴ containing 100 µg of streptomycin sulphate per ml. A single colony arising on this medium was purified twice by streaking minimal medium plus streptomycin. This streptomycin resistant (*str*) derivative of 128c53 was then grown in liquid medium, concentrated, and spread on solid, defined medium containing 100 µg streptomycin and 20 µg rifampicin per ml. A purified single colony resistant to both streptomycin and rifampicin (*rif*) was checked for its ability to nodulate pea seedlings. This *str* and *rif* derivative of 128c53 was then used to isolate five strains of bacteriophage (differing in plaque morphology) from a mixture of mid-western soils according to the procedure of Vincent⁵.

Spontaneously arising mutants of *R. leguminosarum* 128c53 *str* and *rif* which produce decreased amounts of extracellular polysaccharide were obtained by passing late stationary phase cultures of this strain through 1.2-µm Millipore filters, adding an equal volume of fresh medium, and allowing the cells to grow into late stationary phase again. This process was repeated four times over approximately 30 d. These bacteria were then diluted and spread on solid media to observe colony morphology. Over 90% of the colonies arising on such plates were small and seemed to lack the heavy slime produced by the starting strain, 128c53 *str rif*. Seven of these small colonies were purified and examined further.

Table 1 Extracellular polysaccharide production by *R. leguminosarum* 128c53 *str rif*, by EXO-1, and by a revertant of EXO-1, EXO+ 4

Strain	Carbohydrate produced (glucose equivalents ($\text{g} \times 10^{-12}$) per viable count)
128c53 <i>str rif</i>	32.0
EXO-1	1.14
EXO+ 4	31.0

Cells were shaken for 6 d in liquid medium⁴ at 24 °C until a density of approximately 10^8 ml⁻¹ was obtained. Viable counts were then determined by plating appropriate dilutions on solid agar medium⁴. Extracellular material was prepared as follows. Cultures were centrifuged for 30 min at 10,000 r.p.m. The supernatant solutions were passed through 5-µm Millipore filters and then concentrated under reduced pressure and at 40 °C to give one-tenth of the original volumes. The concentrated supernatant solutions were dialysed and lyophilised. The resulting powders were dissolved in 0.2 M imidazole buffer, 0.1 M NaCl, pH 7, and assayed for carbohydrate content by the anthrone method⁶.

Bacteria from all seven small colonies were resistant to streptomycin and rifampicin and sensitive to bacteriophage 1-5 (see above). The strain producing the smallest colonies, designated EXO-1, was chosen for further study.

EXO-1 has a doubling time, both for viable counts and optical density, identical to its parent *R. leguminosarum* 128c53 *str rif* (data not shown). This indicates that EXO-1 does not produce small colonies due to a defect which slows its growth. Extracellular carbohydrate was determined as described in Table 1. On a per cell basis, EXO-1 culture filtrates contain approximately 5% of the amount of anthrone-positive material present in culture filtrates of 128c53 *str rif*. EXO-1 and four similar mutants derived from 128c53 *str rif* by the procedure described above were used to inoculate pea seedlings (Table 2). All five of these strains failed to nodulate Alaska pea.

Indirect evidence suggests that the lipopolysaccharide component of the cell wall of *Rhizobium* may be involved in

Table 2 Nodulation tests

Strain	No. of nodules per plant
<i>R. leguminosarum</i>	280
<i>R. leguminosarum</i> 128c53 <i>str rif</i>	270
None	0
Extracellular polysaccharide Deficient derivatives of <i>R. leguminosarum</i> 128c53 <i>str rif</i>	
EXO-1	0
EXO-2	0
EXO-3	0
EXO-4	0
EXO-5	0
Extracellular polysaccharide revertants of EXO-1	
EXO+ 1	260
EXO+ 2	220
EXO+ 3	230
EXO+ 4	220
EXO+ 5	240

All values are the average of at least three separate experiments, each of which contained at least three plants. Peas were surface sterilised by treatment with 70% ethanol for 5 min followed by treatment with 30% hydrogen peroxide for 30 min. These surface-sterilised peas were soaked overnight in sterile Fahraeus solution⁷ and then planted in sterile vermiculite. The plants were grown using a 14-h light/10-h dark cycle at 22 °C day temperature and 18 °C night temperature. Illumination was 2,500 foot candles. Bacteria were grown to a density of approximately 10^8 ml⁻¹ (ref. 4) by shaking at 24 °C. Cultures were then centrifuged and resuspended in an equal volume of distilled water. Ten-day-old Alaska pea seedlings were inoculated with 10 ml of these bacterial cultures. The roots of these plants were inspected 18 d later. Extracellular polysaccharide producing revertants of EXO-1 were obtained by spreading approximately 10^6 cells of this strain on minimal agar plates⁴. Slime-producing colonies, which arose on these plates at a frequency of approximately 10^{-5} , were isolated and purified.

host-symbiont selection². We therefore examined the lipopolysaccharide of EXO-1 to determine whether this cell surface component is altered by the mutation which reduces extracellular polysaccharide production in this strain. By three criteria, the lipopolysaccharide of EXO-1 seems very similar, if not identical, to that of its parent, 128c53 *str rif*. First, as mentioned above, EXO-1 and 128c53 *str rif* are sensitive to the same bacteriophage. Since most receptors for bacteriophage are located in the lipopolysaccharide⁸, the similarity of phage

Table 3 Glycosyl composition* of the lipopolysaccharides of *R. leguminosarum* 128c53 and 128c53 *str rif* EXO-1

Sugars	<i>R. leguminosarum</i> strains	
	128c53 %	128c53 <i>str rif</i> EXO-1 %
Rhamnose	24	21
Fucose	33	27
Mannose	18	23
Galactose	4	3
Glucose	1	1
2-amino-2, 6-dideoxyhexose	2	3
Uronic acid	14	14
KDO	10	8

Aldoses and amino sugars were analysed as their alditol acetates. The lipopolysaccharides were hydrolysed for 60 min at 121 °C in 2 M trifluoroacetic acid. The resulting monosaccharides were converted to their corresponding alditol acetates and analysed by combined gas chromatography-mass spectrometry¹². 2-Keto-3-deoxyoctanoic acid (KDO) was determined using the thiobarbituric acid colorimetric assay¹³ with the ammonium salt of KDO (Sigma) as the standard. Uronic acid was determined using the *m*-hydroxybiphenyl assay¹⁴ with galacturonic acid as the standard.

*The sugar composition is given as per cent of total sugar. In strain 128c53, the total sugars account for 63% of the lipopolysaccharide mass. In 128c53 *str rif* EXO-1, the sugars account for 27% of the lipopolysaccharide mass.

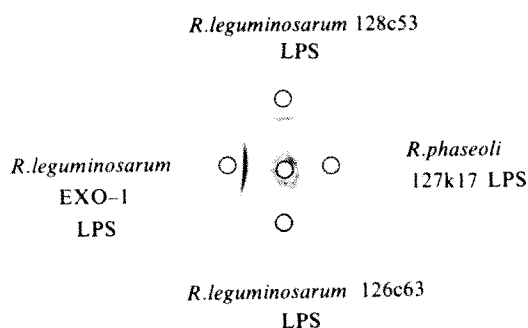


Fig. 1 Immunochemical analysis of the lipopolysaccharides from various *Rhizobium* strains. Antisera (centre well) prepared against the lipopolysaccharide purified from *R. leguminosarum* 128c53 forms a precipitin band when diffused against the lipopolysaccharide from this strain and when diffused against the lipopolysaccharide from its extracellular polysaccharide deficient derivative, EXO-1. The antisera does not cross-react with lipopolysaccharide purified from another strain of *R. leguminosarum* or with lipopolysaccharide purified from a strain of *R. phaseoli*. Each well contains 15 μ l of a 2 mg ml⁻¹ solution of the indicated lipopolysaccharide. In general, the lipopolysaccharides prepared from *Rhizobium* are immunochemically strain and species specific (ref. 9 and R.W.C., C. Napoli, R.E.S. and P.A., in preparation). Lipopolysaccharides were prepared as previously described² with the following modification. The aqueous phase of a phenol-water extract of *R. leguminosarum* 128c53 is viscous and must be ultracentrifuged at 100,000g for 3 h before use of the Agarose A 1.5m column. The other lipopolysaccharide preparations were not as viscous and were purified without the ultracentrifugation step. Before the ultracentrifugation or passage through sizing columns, the lipopolysaccharide preparations were incubated overnight (17 h) at room temperature with 1 mg each of DNase I (Sigma) and RNase A (Sigma) to depolymerise the nucleic acids. The final lipopolysaccharide preparations do not have a λ_{max} between 230 nm and 320 nm and form typical opalescent solutions when dissolved in water at a concentration of 1 mg ml⁻¹. Antiserum to *R. leguminosarum* 128c53 was prepared as previously described^{9,10}. Micro double diffusion tests were performed as described by Crowle¹¹. The diffusion plates (microscope slides) were prepared with 0.75% Noble agar (Difco) in 10 mM sodium phosphate and 0.85% NaCl, pH 7.0. The diffusion tests were allowed to develop for 48 h at 4 °C before being submerged overnight at room temperature in a solution of 10 mM sodium phosphate and 500 mM NaCl, pH 7.0. The plates were then agitated in distilled water for 30 min and stained for 15–30 min in a solution composed of water: isopropanol: acetic acid (13:5:2) containing 0.05% Coomassie brilliant blue R (Sigma). The plates were destained for about 60 min in a solution composed of water: isopropanol: acetic acid (8:1:1).

patterns may be a reflection of lipopolysaccharide structural identity. Second, lipopolysaccharide purified from EXO-1 seems immunochemically identical to lipopolysaccharide isolated from 128c53 *str rif* (Fig. 1). This is particularly pertinent as the lipopolysaccharides of different *Rhizobium* strains are immunologically distinct (R.W.C., C. Napoli, R.E.S. and P.A., in preparation). Finally, the sugar compositions of the carbohydrate moieties of the lipopolysaccharide from EXO-1 and 128c53 *str rif* are very similar as determined by gas chromatography (Table 3).

We have isolated five spontaneous revertants of EXO-1 which have regained the ability to produce copious amounts of extracellular polysaccharide. All five of these revertants have retained resistance to streptomycin and to rifampicin, are sensitive to bacteriophage 1–5, and have regained the ability to nodulate pea seedlings (Tables 1 and 2). In these respects, all five of these revertants seem identical to the starting strain 128c53 *str rif*. It therefore seems likely that a single mutational event is responsible for both the reduced production of extracellular polysaccharide by EXO-1 and the inability of EXO-1 to nodulate pea seedlings.

A simple hypothesis which accounts for the above data is that the reduced production of extracellular carbohydrate by the mutant EXO-1 is related to the inability of this strain to nodulate pea seedlings. Further analysis of EXO-1 and

additional mutants affecting the surface polysaccharides of *Rhizobium* may confirm that, as in other systems^{15,16}, surface polysaccharides participate in the infection of eukaryotes by prokaryotes.

This work was supported by the US NSF (grant PCM75-13897) and the Rockefeller Foundation (RFGAAS 7510). R.E.S. thanks the Helen Hay Whitney Foundation for financial support.

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Received 12 September; accepted 7 November 1977.

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Mutations to increased antibiotic sensitivity in naturally-occurring gonococci

CLINICAL isolation of mutations giving increased drug sensitivity has not been previously reported. The experiments presented here indicate that a significant proportion of clinical strains of *Neisseria gonorrhoeae* contain mutations which result in markedly increased susceptibility to a variety of antibiotics. Previously, Maness and Sparling¹ isolated and characterised mutants (*env*) of laboratory gonococci which had markedly increased sensitivity ('hypersensitivity') to a variety of antibiotics, detergents and dyes. The *env* mutations were phenotypically dominant over, and totally suppressed, low-level nonspecific drug resistance determined by mutations at a locus formerly designated *ery*², but now designated *mtr*³. Both *mtr* and *env* affected the permeability of the cell envelope⁴. Most of the clinical hypersensitive strains are also shown to possess mutations to nonspecific drug resistance, similar to *mtr*, which are suppressed phenotypically. We have studied strains which included 502 random isolates from patients in North Carolina with uncomplicated gonococcal urethritis and cervicitis, as well as selected erythromycin-hypersensitive strains from London (kindly supplied by I. Phillips), and five penicillinase-producing gonococci (four from the Far East, one from Rotterdam).

Sarubbi *et al.*² have shown that *env* mutants are at least four times more sensitive to erythromycin, fusidic acid, crystal violet (CV) and Triton X-100 (Trx) than are wild-type strains. Accordingly, we determined the minimal inhibitory concentrations (MICs) to erythromycin for all isolates, using an agar dilution method⁵. Wild-type strains, without *env* or *mtr*, generally are inhibited by 0.25–1 μ g ml⁻¹ Ery². Clinical isolates with erythromycin MIC \leq 0.12 μ g ml⁻¹ were considered possible *env* mutants, and were further evaluated for sensitivity to fusidic acid, crystal violet and Triton X-100. Isolates hypersensitive to all drugs were studied for the presence of *env* and suppressed *mtr* mutations by genetic transformation, employing standard recipient strains and methods^{6,7}. A hypersensitive clinical isolate was considered to contain a suppressed *mtr* mutation if it was

capable of donating a gene for nonspecific low-level resistance to erythromycin, fusidic acid and Triton X-100 to wild-type strain FA19^a.

Clinical *env* mutants were then further characterised as to the type of *env* mutation they possessed by transformation crosses with laboratory strains BR54 (*env*-3) and BR87 (*env*-2). The *env*-2 and *env*-3 mutations are known to occur at separate sites based on recombination frequencies in transformation crosses². To facilitate genetic analysis of the *env*-mutations, high-level rifampin resistant (Rif^R) mutations were selected from the clinical *env* mutants. DNA prepared from such strains were used to transform BR87 and BR54, selecting separately for Rif^R and Env⁺. The Rif^R transformants served as an internal control of the efficiency of the transformation system. The recombination index² (ratio of Env⁺ to Rif^R transformants) was used to determine the proximity of the clinical *env* mutations to *env*-2 and *env*-3.

Results showed that 54 of 347 strains isolated in 1973 (16%) and 30 of 155 strains from 1976 (19%) were hypersensitive to erythromycin (MIC $\leq 0.12 \mu\text{g ml}^{-1}$). Of 35 of these strains further evaluated, 32 were hypersensitive to fusidic acid ($\leq 0.03 \mu\text{g ml}^{-1}$), crystal violet ($\leq 2 \mu\text{g ml}^{-1}$) and Triton X-100 ($\leq 0.25 \text{ mg ml}^{-1}$), whereas none of 53 evaluated wild-type or erythromycin resistant strains were hypersensitive to any of the other drugs; three of five penicillinase-producing gonococci were hypersensitive to erythromycin, fusidic acid, crystal violet and Triton X-100.

Fifteen of 16 tested hypersensitive strains, including the three penicillinase-producing isolates, donated a suppressed gene phenotypically identical to the previously described *mtr* mutation to wild-type FA19. In a typical experiment, a hypersensitive (erythromycin MIC = $0.06 \mu\text{g ml}^{-1}$) donor transferred to the wild-type (erythromycin MIC = $0.25 \mu\text{g ml}^{-1}$) recipient a gene for low-level ($4.0 \mu\text{g ml}^{-1}$) resistance to erythromycin; all Ery^R transformants were also much more resistant to fusidic acid, Triton X-100 and other drugs. Most phenotypically hypersensitive clinical isolates were therefore genotypically resistant.

Transformation crosses between hypersensitive clinical isolates and the isogenic laboratory strains BR54 (*env*-3) and BR87 (*env*-2) confirmed that most of the clinical hypersensitive isolates were *env* mutants (Table 1). In the initial experiments, donor DNA from the clinical isolates was introduced into BR54 and BR87. Results showed that IP6 recombined with much higher frequency with BR87 (*env*-2) than with BR54 (*env*-3), and thus probably contains an *env* mutation very close to *env*-3. FA3024, as well as the penicillinase-producing isolates FA290 and FA362, recombined with high frequency with BR54 but with only very low frequency with BR87. In other experiments, 11 additional hypersensitive strains behaved like FA3024; thus 14 of 16 studied strains were apparently mutant at a site close to *env*-2. In contrast D1519 recombined with both laboratory strains, as well as IP6 and FA3024, and therefore is apparently mutant at a site distinct from those described previously. Reciprocal experiments in which *rif*-2 derivatives of the isogenic set FA19 (*env*⁺), BR54 (*env*-3) and BR87 (*env*-2) were used as donors, and the hypersensitive clinical isolates as recipients, gave very similar results (Table 1).

We suggest that approximately 15% of random clinical isolates from North Carolina, as well as three of five penicillinase-producing isolates, contain *env* mutations, most of which occur in the same gene as the previously characterised *env*-2 mutation, which has recently been shown to result in altered outer membrane proteins by the method of SDS-polyacrylamide gel electrophoresis (D. Walstad, L. Guymon, & P.F.S., unpublished). Presumably the *env* mutation confers on to these strains some selective advantage which cannot be related to their ability to grow in the presence of antibiotics, as these strains are unusually sensi-

Table 1 Recombination indices (Env⁺/Rif^R) in transformations between clinically-isolated antibiotic-hypersensitive (*env*) gonococci and isogenic laboratory mutants containing known *env* mutations*

Donor strains†	Recipient strains‡				
	BR54 (<i>env</i> -3)	BR87 (<i>env</i> -2)	FA3024 (<i>env</i> -5)	FA290 (<i>env</i> -7)	FA362 (<i>env</i> -8)
IP6 <i>rif env</i> -4	0.006	0.4			
FA3024 <i>rif env</i> -5	0.5	0.0005			
FA290 <i>rif env</i> -7	1.2	0.02			
FA362 <i>rif env</i> -8	1.9	0.03			
D1519 <i>rif env</i> -9	0.7	0.2			
FA19 <i>rif env</i> ⁺	2.0	1.5	2.3	1.2	1.5
BR54 <i>rif env</i> -3	< 0.0001	2.3	1.5	0.9	5.0
BR87 <i>rif env</i> -2	1.2	< 0.0001	0.01	0.0009	0.02

*Recombination index equals the number of Env⁺ transformants ml⁻¹ (selected with erythromycin at a concentration of $0.25 \mu\text{g ml}^{-1}$) divided by the number of Rif^R transformants ml⁻¹ (selected with rifampin at a concentration of $2 \mu\text{g ml}^{-1}$). Results are means of at least two experiments. DNA concentrations were saturating.

†All clinical donor strains (IP6, FA3024, FA290, FA362, D1519) were made rifampin resistant (Rif^R) by purifying spontaneous mutants from plates containing $5 \mu\text{g ml}^{-1}$ rifampin. The isogenic laboratory strains (FA19, BR54, BR87) were made Rif^R by introduction of the *rif*-2 allele by transformation.

‡IP6 and D1519 were not competent, and therefore could not be used as recipients.

tive to antibiotics.

In the laboratory these strains autolyse more readily⁴ and would, therefore, appear to be at a disadvantage. We have, however, noted that with laboratory strain FA19, the *mtr* mutation consistently results in reduced rates of exponential growth in enriched broth cultures, whereas introduction (by transformation) of an *env* mutation into the derivative carrying *mtr*, FA140, results in partial restoration of normal growth rates *in vitro* (Table 2). Similar results occurred with the clinical *env* strain FA3024 and its *env*⁺ derivative FA507. We have no evidence, of course, that similar growth rates occur *in vivo*, and other explanations such as greater mucosal adherence of *env* mutants are conceivable.

In the pre-antibiotic era, tested strains of *Neisseria gonorrhoeae* possessed neither low-level resistance nor hypersensitivity to antibiotics⁵. The pressure of antibiotic usage has selected strains with low-level resistance over the past three decades. The commensurate poorer growth characteristics of some of these mutants, though, might have resulted in selective advantage for subsequent mutations which improved growth and decreased resistance. Paradoxically,

Table 2 Log phase doubling times for *env* and *env*⁺ strains

Strain	Ery MIC ($\mu\text{g ml}^{-1}$)	Doubling Time (min) [†]	P Value [‡]
Isogenic laboratory set*			
FA19 (wild type)	0.25	52.7 \pm 4.66	> < 0.005
FA140 (<i>mtr</i>)	4.0	69.3 \pm 2.97	
BR87 (<i>mtr, env</i>)	0.06	57.4 \pm 5.78	
Isogenic clinical set			
FA3024 (<i>mtr, env</i>)	0.06	49.9 \pm 4.27	> < 0.005
FA507 (<i>mtr</i>)	4.0	76.5 \pm 6.99	

*Parent strain FA19 and its transformation derivatives have been described by Sarubbi *et al.*². Both FA140 and BR87 contain other mutations besides those shown², but the slow growth of FA140 is due almost entirely to the *mtr* mutation (data not shown). The full genotype of the clinical isolates is not known.

†Mean $\pm \sigma$. Cells were grown in GC base broth with added supplements². Growth was at 37°C in 125 ml side-arm flasks which were agitated in a metabolic shaker with 5% CO₂. The amount of growth was determined by optical density on a Klett-Summerson colorimeter (No. 540 filter).

‡Paired Student's *t* test.

antibiotic usage may be selecting gonococci with both greater antibiotic resistance and, indirectly, greater sensitivity.

We thank D. Walstad for assistance, and I. Phillips, C. Thornsberry and E. Stolz for donating strains. This work was supported by a postdoctoral fellowship from the USPHS to B.I.E. and NIH research grant to P.F.S.

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Received 23 June; accepted 24 November 1977.

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Control of microbial surface-growth by density

In synchronous cultures of bacteria, the rate of cell elongation¹ or of envelope synthesis² seems to increase abruptly at a particular age. These and other findings suggest³⁻⁵ that bacterial envelope is formed at a constant rate, possibly by enzymes organised in zones, and that some event in the cell cycle leads to a discrete increase in the amount of enzymes or number of zones concerned. (The actual event involved has been variously identified with initiation of chromosome replication⁴, with termination^{1,5} and with the attainment of a critical cell length⁶.) Growth zones are known to occur in the filamentous fungi⁷. The rate at which these zones are able to add new envelope is limited, and since mass increases exponentially⁸, hyphal density eventually begins to rise; when it reaches a critical value, new zones are formed. Thus density (or indeed the concentration of any product synthesised exponentially⁹) could control the rate of surface growth, and we suggest that such is the case in bacteria.

We postulate a constant rate of surface-area synthesis β that doubles once during the bacterial cycle, d min before division¹⁰. For a cylinder with open ends, the cell volume $V(a)$ at age a is given by

$$V(a) = 2^{m-1} R\beta\tau [1 + (a+d)/\tau - m] \text{ for } a \leq (m+1)\tau - d \\ = 2^m R\beta\tau [(a+d)/\tau - m] \text{ for } a \geq (m+1)\tau - d \quad (1)$$

where τ is the mean doubling time of the cell, R is the radius, and $m \equiv [d/\tau]$. If we define ρ_d as the density of the cell at age $\tau-d$, then

$$\rho_d \equiv (M_d/V_d) = (M_1/R\beta\tau)2^{(C+D-d)/\tau} \quad (2)$$

where M_d and V_d are the mass and volume at age $\tau-d$. Here C and D are the times taken to replicate the chromosome and to complete division processes, respectively¹¹, and M_1 is the (constant) ratio of cell mass to chromosome origins at the time of initiation of replication¹².

There is reliable experimental evidence that R does not vary very much over the cell cycle during steady-state growth¹³. If we take it to be completely independent of a , then

$$\bar{V} = (M_1/2\rho_d \ln 2)2^{(C+D)/\tau} \quad (3)$$

where \bar{V} has been defined in the usual way as

$$\bar{V} = \int_0^\tau V(a)v(a)da / \int_0^\tau v(a)da,$$

and $v(a)$ is the frequency function of age.

Because ρ_d is considered to be the critical density that leads to the addition of envelope growth-zones, one would expect it to be independent of τ ; \bar{V} should thus vary with τ according to the exponential term. We have tested this prediction against the dimensions of *Escherichia coli* B/r strain H266 growing with a variety of doubling times (Table 1). Non-linear regression analysis to fit equation (3) to average cell volume gave excellent results ($P < 10^{-6}$ from a two-tailed F -test of explained variance). The value obtained for $C+D$ (78.7 ± 7.6 min) is so close to accepted values¹⁴ as to preclude the possibility that ρ_d is exponentially dependent on the growth rate to any great extent. It should be stressed that equation (3) has been derived without any assumptions concerning the dependence of β , R or d on τ . Furthermore, the proportionality of \bar{V} to $2^{(C+D)/\tau}$ found here may also hold true for other Gram-negative bacilliform bacteria. This follows from the similar dependence of the average mass of *Salmonella typhimurium*^{12,15} on $(C+D)/\tau$ and the independence¹⁶ of the average cell density ρ .

In addition to a maximum at $\tau-d$, cell density goes through a minimum during each cycle. This minimum precedes $\tau-d$ by a fixed fraction of τ , $2-1/\ln 2$ or about 56%, and will thus occur after, at or before cell division according as $d/\tau <, =$ or $> m-1+1/\ln 2$; in all cases, the ratio ρ_{min}/ρ_d is constant and equal to $\frac{1}{2}\ln 2 \approx 0.94$. (By comparison, ρ/ρ_d , which is also independent of τ , is $2(1/\ln 2)^2 \approx 0.96$.)

Evidence¹⁷ for just such a variation in ρ over the cell cycle has recently been reported for exponentially growing *E. coli* K12, with measured values of 0.95 for ρ_{min}/ρ_d and 0.97 for ρ/ρ_d ; ρ/ρ_{min} was precisely as predicted, 1.020. The occurrence of the maximal density at cell birth and division is consistent with a d of around 44 min; the least-squares estimate provided by the surface-area model (Rosenberger *et al.*, submitted for publication) is 49 ± 4 min. The location of the minimal density, near the middle of the cell cycle, is also as expected.

The time before division at which the rate of envelope synthesis doubles, d , will depend on R and β and the way they vary with τ . The radius of *E. coli* B/r H266 decreases with τ (Table 1): over 90% of the variance ($P < 10^{-6}$) in $R(\tau)$ can be accounted for by the expression $R(\tau) = R_\infty 2^{1/\tau}$, where $R_\infty = 0.185$ μm and $\gamma = 33.6$ min. In order to define the relationship of d to growth rate, it is thus only necessary to determine how β varies with τ . In the absence of definitive data, two simple relationships are considered¹⁰ (see also Rosenberger *et al.*, submitted for publication): no dependence between β and τ , and inverse proportionality. The former predicts d to be a weak function of τ ; the latter to be a constant, $C+D-\gamma$, about 45 min. In either case, and depending on the growth rate, d could coincide with either the initiation or the termination of chromosome replication. This may explain the general lack of agreement in the literature regarding the particular event in the cell cycle considered to be associated with the rate change in envelope synthesis^{1,5,6}.

The dimensions predicted by models postulating linear and exponential rates of envelope synthesis are very similar¹⁸. We would thus like to point out that exponential surface extension between discrete changes in the number of zones can also explain the experimental results, as follows. If the rate of surface growth from a zone increases in proportion to cell mass until the zone reaches its maximum capacity, as is the case in fungi⁷, then cell density will be constant except for the period when all existing zones are acting at full capacity and new ones have not yet become operative; provided this period is short compared to τ , \bar{V} will still be proportional to $2^{(C+D)/\tau}$.

While there seem to be no recognised molecular mechanisms through which changes in density are able to derepress enzyme synthesis, changes in solute concentrations can do so.

Table 1 Mean length \bar{L} and radius R of *E. coli* B/r at various doubling times τ

Medium containing	τ (min)	\bar{L} (μm)	R (μm)
Alanine	160	2.39	0.217
	160	2.20	0.212
	160	2.19	0.212
	124	2.72	0.238
Succinate	105	2.27	0.250
	72	2.64	0.229
Alanine+proline	72	2.80	0.288
	60	2.53	0.232
Glycerol	45	2.62	0.308
Glucose	45	2.66	0.300
	45	2.68	0.312
	32	3.14	0.321
	31	3.35	0.446
Glucose + casamino acids (Difco)	31	3.08	0.406
	31	3.07	0.417
	31	3.22	0.429
Glucose + casein Hydrolysate (Sigma)	24	3.58	0.479
	24	3.29	0.466

E. coli B/r strain H266 was grown in minimal salts medium supplemented with the carbon source indicated¹⁰. Cells, fixed in OsO_4 and agar-filtered, were measured from projections of electron micrographs at a final magnification of 12,000.

If the limiting precursor of an envelope component were synthesised with the same kinetics as is mass, its concentration too would reach a maximum at $\tau - d$; for a relatively large flux and low average concentration, such an increase could be quite substantial.

One should perhaps consider another possibility: that cell radius varies during the cell cycle in such a way as to maintain p constant. Of course, if the cell wall were completely free to adjust to changes in hydrostatic pressure, R rather than p would reach a maximum at $\tau - d$. Here too, the maximum would be only 4% above the average value and 6% above the minimum—variations that are probably beyond experimental resolution¹³. But cell wall, once formed, is able to withstand considerable internal pressures and yields very little (if at all) to forces of expansion. Mitchell¹⁹ measured the phosphate-impermeable volume of stationary *E. coli* as a function of external NaCl concentration in conditions that were later found to cause a substantial cleavage of the peptides crosslinking murein chains²⁰. Nevertheless, less than half the increase in internal osmotic pressure was compensated for by a change in cell volume, the remainder being balanced by the hydrostatic pressure exerted by the cell wall²¹. In principle, in the case of growing cells, new cell wall could attain the necessary magnitude as it is being synthesised in order to maintain constant density despite the rigidity of formed wall. If we assume, as a conservative upper limit, that formed wall can adjust sufficiently to compensate for half the change in volume required to keep p constant, then one can show (and verify easily) that in order for new wall to compensate for the other half, the radius must increase exponentially along the length of the cell from a value of R_0 ($= 2M_0 \ln 2 / \beta p \tau$, where M_0 is the mass at birth) at the location corresponding to cell birth to a maximum of $2R_0 2^{-d/\tau}$ at doubling. There it drops abruptly to half its size and then resumes its exponential climb back to R_0 . Such behaviour is clearly at variance with observation, and we must reject the possibility of constant p and adjustable R .

The applicability of our model will depend in part on the extent to which it can explain the differences in shape observed in closely related strains of *E. coli* growing in a variety of conditions^{5,6,10,14,16,22}, including those produced by specific mutations^{5,23,24}. In this connection, the bizarre alterations of shape found in *E. coli* 15T⁻ growing rapidly in low thymine concentrations²⁵, may be of particular interest.

We thank R. H. Pritchard and E. Orr for valuable discussions. This work was supported in part by the Israel Academy of

Sciences, Commission for Basic Research (to A.Z.) and an EMBO short-term fellowship (to C.L.W.).

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Received 19 April; accepted 15 November 1977.

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Suppression of fever at term of pregnancy

THE newborn human is often unable to develop a fever following exposure to various infectious agents^{1–3} and newborn lambs are similar, in that they do not develop a fever in response to a bacterial endotoxin challenge in the first few days of life⁴. Lack of the febrile response is thought to be due to the need for a process of 'sensitisation' of the lambs, or to the immaturity of some aspect of the fever-production process^{5,6}. Newborn guinea pigs and rabbits also, do not become febrile or show a diminished response to a challenge with endotoxin during early postnatal life^{7,8}. We have examined the response of the pregnant ewe to pyrogens at and near the time of birth, for although they become febrile during pregnancy⁹, their response to endotoxin at term has not been studied. We report here that the febrile response induced by a bacterial endotoxin or by endogenous pyrogen is suppressed in the ewe for a period extending from 2 to 5 d prepartum to several hours postpartum.

Suffolk, Dorset and cross-bred ewes were studied at various times ranging from 8 d prepartum to 60 h postpartum. The ewes were injected by way of the jugular vein with 30 μg of bacterial pyrogen derived from *Salmonella abortus equi* (SAEP) in a 3-ml volume of sterile physiological saline. This quantity of SAEP gave a fever of $1.3 \pm 0.15^\circ\text{C}$ in non-pregnant adult sheep. The SAEP was administered to each ewe at 3-d intervals, in order to avoid the production of refractoriness to the pyrogen. Temperatures were measured continuously by a thermistor probe inserted about

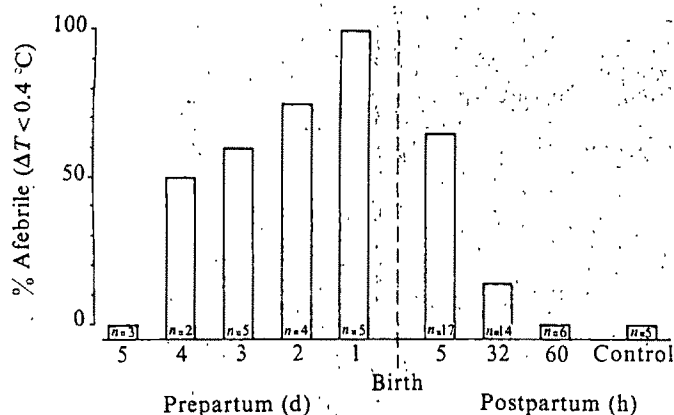


Fig. 1 The proportion of ewes not responding with a fever following 30 μ g SAE pyrogen given intravenously is represented by the vertical bars. The animals were injected from times ranging from 5 d prepartum to 60 h postpartum. The responses of non-pregnant adult ewes is represented on the far right. The broken vertical line represents the time of birth.

20 cm beyond the anal sphincter and the output of the thermistor was displayed on a recorder. Rectal temperature was monitored until it had remained steady for 30 min before injection and then was monitored for 200 min after injection. A fever was considered to be a 0.4 °C or greater rise in temperature above that at the time of injection.

Endogenous or leukocyte pyrogen (EP) was prepared from sheep granulocytes, obtained by a sterile peritonitis induced with 3% starch solutions after the method of Fessler *et al.*¹⁰ This EP was found to be endotoxin-free using Murphy's rabbit test¹¹ and by the Limulus assay for Gram-negative material^{12,13}. A fever with a maximum of 1.85 ± 0.10 °C was produced by 0.2 ml of this EP in the non-pregnant adult sheep. The EP was injected in the same manner as was the SAE after it was found that the ewes did not respond to SAE with a fever. This procedure was intended to determine at which step in the sequence of events of fever production the inhibition was manifest, since EP is thought to be released from cells of the reticuloendothelial system once they are stimulated by the endotoxin or antibody-endotoxin complex. It is thought that EP stimulates some higher centre(s) which react by driving heat production and heat conservation.

The ewes responded with fever to SAE until a time which varied between 4 and 2 d prepartum. As shown in Fig. 1, 5 d before parturition, all the three ewes tested developed a fever similar to that in the non-pregnant controls. At 3 and 4 d before parturition four of seven ewes were afebrile following the injection of pyrogen. At days 1 and 2 prepartum, eight of the nine ewes tested failed to develop a fever. The ewes were injected with SAE 5 h postpartum, and 11 of 17 remained afebrile, whereas at 32 h postpartum, 12 of 14 ewes could again become febrile. By 60 h, all the six ewes tested were febrile. Most EP challenges fell within the 0–48 h prepartum period, as they were given after the ewe became afebrile to SAE. Although the number of experiments using EP is small, it was observed that four of seven ewes, 1 and 2 d prepartum responded to EP with fevers whereas three were afebrile. It was not possible during this breeding season to do additional experiments with EP at other times. All control animals responded with fever following every injection of both SAE and EP.

These data suggest the presence of some naturally occurring fever-suppressing system. First, there could be a humoral antipyretic agent which is shared by the circulation of both ewe and foetus. This is compatible with the observation that the newborn lamb also does not become febrile to either SAE or EP. Second, it could be possible that the ewe is afebrile because of a circulating antipyretic but the newborn lamb, on the other hand, is afebrile as the result of some immature cellular system in the fever pathway. The fact that the EP never produced a fever in the newborn animal suggests that it may be afebrile by some other mechanism or, consistent with the first possibility, that the titre of antipyretic

substance is much higher than in the ewe. Injections of EP were given in order to determine at which step in the sequence of events inhibition is occurring within the system. The differential response to the EP among the prepartum ewes, four febrile and three afebrile, at 0–48 h before birth, suggests that the inhibition occurred after EP had been elaborated^{11–14}. An explanation consistent with this is that with our dose of EP, the titre was higher than that naturally produced by the reticuloendothelial system in response to 30 μ g SAE. Since EP produced a fever in four of seven animals, it is likely the inhibition was at some stage after the elaboration of EP and raises the possibility that higher doses of EP may overcome this inhibition. This explanation requires that inhibition is not total but that the putative antipyretic acts by competitive inhibition of EP or some later mediator of fever.

The evidence presented here that there is a suppression of fever near term, would indicate some adverse consequences of fever at term. The ewes which produced fevers to EP during their critical period, however, showed no more trouble with parturition than the afebrile mothers. The lambs of EP febrile mothers were no less viable than those of EP afebrile mothers, judging from observations such as ability to suckle soon after birth, strength and growth rate.

It remains to be seen if term ewes can become febrile to any pyrogenic agent other than EP or if any other mammals, particularly human, show in some way this endogenous antipyretic phenomenon. The identity of such an antipyretic is another critical question and its characterisation could add significantly to our understanding of the febrile process and antipyresis.

Supported by the Medical Research Council of Canada. N.W.K. is a predoctoral student of the MRC. Endotoxin assays were carried out by G. Mears.

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Received 26 September; accepted 14 November 1977.

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Action of *Corynebacterium ovis* exotoxin on endothelial cells of blood vessels

Corynebacterium ovis (*C. pseudotuberculosis*) causes suppurative infections of sheep, goats and horses and occasional infections in other species². Like *C. diphtheriae*, it produces a powerful exotoxin *in vitro*. But unlike the situation with *C. diphtheriae*, there is no evidence of acute or chronic intoxication, while the lesions are characteristically pyogenic with suppuration and abscess formation at the initial site of infection (usually a skin wound). This is followed frequently by secondary abscess formation in regional lymph nodes and sometimes internal organs. Toxin is produced *in vivo* as well as *in vitro* because circulating antitoxin is found in infected animals. The major role of *C. ovis*

toxin in natural infection seems to be facilitation of the spread of the causative bacteria by its action as a permeability factor. It thus causes marked leakage of plasma from small blood vessels at the site of infection which floods lymphatic spaces and increases the risk of bacteria being carried by lymphatic drainage from the site of infection to regional lymph nodes^{3,4}. We now report experimental evidence that *C. ovis* toxin is a phospholipase D which attacks the sphingomyelin of endothelial cells of blood vessels.

We considered two possible modes of action for *C. ovis* toxin. The first was an indirect effect in which toxin acts on mast cells closely associated with blood vessels, leading to liberation of histamine and 5-hydroxytryptamine which then act on adjacent endothelial lining membranes of blood vessels, increasing their permeability. The second was a direct action of the toxin molecule on endothelial cells. When mast cells from the peritoneal cavity of rats and mice were exposed to toxin, no degranulation was observed. This accorded with the observations of Smith and Miles⁵ who found no evidence of liberation of histamine or 5-hydroxytryptamine when *C. ovis* culture was introduced into the peritoneal cavity of the rat.

Souček *et al.*^{6,7} have reported that purified *C. ovis* toxin is a phospholipase D which splits sphingomyelin into *N*-acyl-sphingosyl (ceramide) phosphate and choline. This action occurred both when purified sphingomyelin was used as substrate and when the lipid was incorporated in the lipoprotein of the cell membrane of erythrocytes. This suggested that *C. ovis* toxin acts in a similar manner on the sphingomyelin of endothelial cells lining blood vessels. We have examined this by exposing living endothelial cells lining the aorta of sheep and rabbits to purified *C. ovis* toxin. We then examined chloroform:methanol extracts of the treated cells for evidence of ceramide phosphate and choline—the breakdown products of sphingomyelin by a phospholipase D. Washed sheep and rabbit erythrocytes were also exposed to toxin for comparison.

Toxin was precipitated from culture filtrates by 35–65% saturation with ammonium sulphate. This was followed by ion-exchange chromatography on a column of Sephadex CM 50, pressure dialysis and freeze drying. (Further purification has recently been achieved by use of DEAE columns, but the biological properties have remained essentially the same. Details will be published elsewhere.) A solution of 1 mg ml⁻¹ in phosphate-buffered saline (PBS) had a minimal reactive dose in the rabbit of 0.2 ml of 10⁻⁵ dilution. The aortae of five sheep and two rabbits were exposed to toxin (1 mg ml⁻¹ in phosphate-buffered saline containing 4 mM MgCl₂); and as controls, aortae of two sheep and one rabbit were similarly treated either with toxin inactivated by heating at 60 °C for 2 h, or buffer only. Animals were anaesthetised and most received heparin intravenously to prevent clotting. After bleeding out, the abdomen and thorax were quickly opened, cannulae were inserted into the aortic arch and near the bifurcation of the abdominal aorta; the aorta was then perfused with PBS while all arterial branches were ligated and severed. The aorta was removed, filled with toxin or control fluid, suspended in PBS and held at 37 °C for 1–2 h. Phospholipids of the endothelial lining were then extracted by chloroform:methanol (2:1 v/v) based on the method described by Dawson *et al.*⁸. Erythrocytes from 50 ml of heparinised blood were washed three times in PBS containing 4 mM MgCl₂. One-half of the deposited cells were mixed with toxin (1 mg ml⁻¹) and the other with heat-inactivated toxin. Suspensions were incubated at 37 °C, and cells were finally extracted with chloroform:methanol (2:1).

Equal volumes (200 µl for aortae, 150 µl for erythrocytes) of control and treated extracts were spotted on to thin-layer chromatography (TLC) plates (Merck silica gel 60F-254). Sphingomyelin and ceramide phosphate were included as markers. TLC plates were developed with chloroform-methanol-water-acetic acid (65:50:4:1 by volume). The phospholipids were detected by a phosphorus-specific spray⁹. Some TLC plates were sprayed with a choline-detecting reagent¹⁰.

In all extracts of toxin-treated aortae and erythrocytes of both

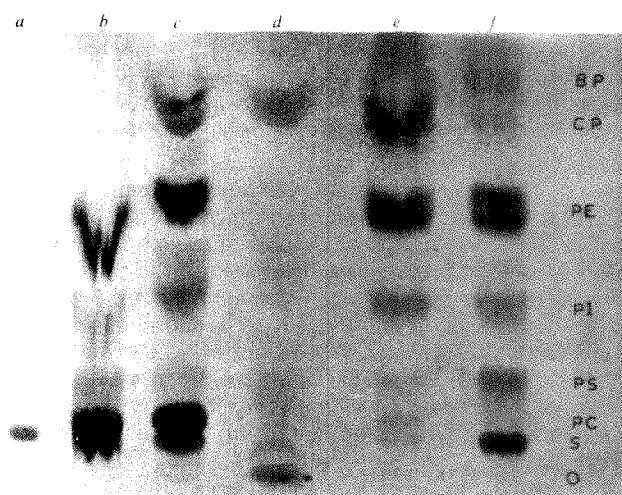


Fig. 1 TLC plate extracts of toxin-treated and control aortic endothelium and erythrocytes of sheep. Vertical lanes: a, sphingomyelin; b, control aortic extract; c, toxin-treated aortic extract; d, synthesised ceramide phosphate; e, toxin-treated erythrocytes extract; f, control erythrocytes extract. Horizontal lanes: BP, blood pigments; CP, ceramide phosphate; PE, phosphatidyl ethanolamine; PI, phosphatidylinositol; PS, phosphatidyl serine; S, sphingomyelin; O, origin.

sheep and rabbits, sphingomyelin was degraded to ceramide phosphate and choline. Figure 1 shows a TLC plate of the sheep extracts sprayed for phospholipids.

After completion of these experiments our attention was drawn to reports that certain enzymes, including phospholipases, may not be inactivated by chloroform:methanol used to extract tissues. If this were the case with *C. ovis* toxin, then the ceramide phosphate demonstrated in our extracts of aortic endothelial cells and erythrocytes may not have resulted from direct action of toxin on sphingomyelin bound to protein in the endothelial cell membrane, but may have occurred as the result of phospholipid degradation by still active toxin after extraction by organic solvent. We therefore set up the following tests. (1) We mixed 0.5 ml of emulsion of sphingomyelin in water with 0.5 ml of toxin (1 mg ml⁻¹ in phosphate-buffered saline + MgCl₂); added 20 ml of chloroform:methanol (2:1 v/v), incubated them together for 2 h at 37 °C and ran the mixture on TLC plate. (2) As for (1) but the mixture was tested on a TLC plate immediately after mixing. (3) and (4) as for (1) and (2) but a suspension of washed sheep erythrocytes was substituted for sphingomyelin emulsion. In each case phospholipase D activity of *C. ovis* toxin was annulled by exposure to the chloroform:methanol, even for a few minutes.

We have thus confirmed (the evidence of Souček *et al.*^{6,7}) that *C. ovis* toxin is a sphingomyelinase which splits the sphingomyelin of the cell membrane of erythrocytes into ceramide phosphate and choline, but we have extended our experimental observations to show that the toxin has a similar action on the sphingomyelin of the vascular endothelial membrane. This raises the question of the possible significance of this enzyme action of *C. ovis* toxin in relation to its permeability effect on blood vessels.

It is interesting that *C. ovis* toxin produces its permeability changes rapidly within minutes. This seems to exclude the possibility that this early action was due to permeability factors derived from leukocytes or platelets which are not seen to adhere to the luminal surface of endothelial cells until 30 min or longer after injection of toxin.

In spite of extensive study, little is known of the biochemical and biophysical causes of increased vascular permeability which is such a striking feature of acute inflammation and the action of certain bacterial toxins^{11–14}.

Majno *et al.*^{15,16} have shown that exposure of small blood vessels to the well-known permeability agents, histamine and 5-hydroxytryptamine, leads to partial disconnection between

endothelial cells along intercellular junctions, thus forming gaps through which leakage of plasma occurs. Among possible factors leading to the formation of such gaps may be the chemical breakdown of specific junction constituents (for example, the protein connexin isolated by Goodenough¹⁷ from junctions between mouse liver cells), and/or increased tension on junctions resulting from contraction of the cell membrane caused by chemical changes in important constituents over the whole cell surface. Sphingomyelin, which is broken down by *C. ovis*, forms part of an important structural lipoprotein of the cell membrane. It is interesting that the alpha toxin of *Clostridium welchii*, another active permeability agent, is a phospholipase C and attacks lecithin, the phospholipid component of another important structural lipoprotein of cell membranes¹⁸.

We are examining the possible significance of the enzyme action of *C. ovis* toxin on sphingomyelin in relation to its permeability effect on blood vessels.

We thank Dr R. M. C. Dawson for advice; Dr D. E. Bowyer, P. E. Davies and M. A. Reidy for helpful discussions; Mr G. A. Embleton and Dr R. M. Moor for anaesthesia and facilities; Mr C. R. Farrell and Mrs J. P. King for technical assistance, and Mr P. C. Curtis for photography. We also thank Professors R. I. N. Greaves and P. Wildy for hospitality and facilities in the Department of Pathology, University of Cambridge. This work was supported by a grant from the Wellcome Trust.

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Received 16 June; accepted 14 November 1977.

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Differential cell adhesion may result from nonspecific interactions between cell surface glycoproteins

I SUGGEST that if different cell types have different glycoprotein coatings they will tend to adhere to each other with different intensities, and sort out into separate tissues simply as a result of weak nonspecific interactions between the glycoproteins without any special mechanisms such as binding by specific recognition proteins. This could be responsible for many, but certainly not all, cell recognition and adhesion phenomena (reviewed in refs 1 and 2).

Cells are coated with glycoprotein and glycolipid so they would seem from the outside like giant macromolecules of glycoprotein (for simplicity, the glycolipid can be pictured as providing extra oligosaccharide chains in this glycoprotein) and they ought to show behaviour analogous to the behaviour of large polymers in solution.

Large polymers when mixed in solution tend to undergo incompatible phase separation, the solution separating into immiscible phases each containing predominantly one polymer

species³. For example, a sufficiently concentrated solution of polyethylene glycol and dextran separates into two phases, and many other polymers, when added to the system, will form further separate phases. Two separate phases can even form from solutions of one polymer and a small molecule, for example polypropylene glycol and potassium phosphate or glucose³. The behaviour of small molecules in solution is usually determined mainly by the entropy of dilution, representing random distribution of the solute throughout the solvent. For polymers the entropy of dilution remains, to a first approximation, the same per molecule but the enthalpy of interactions between the polymer molecules increases in proportion to the size of the molecules and at sufficiently high molecular weight may become the dominant influence. For hydrophilic polymers these interactions will mainly be weak and non-specific, such as hydrogen-bonding, van der Waal's, fixed dipole-dipole and hydrophobic interactions. Such interactions are generally stronger between like molecules than between unlike molecules, so large polymers tend to segregate into separate phases⁴. (There are, of course, many polymers, particularly charged ones, that do interact with each other more strongly than with themselves. They may form complexes that separate as a single phase from solution leaving a polymer-free phase.) An additional factor promoting segregation of unlike molecules arises from steric effects. Each polymer molecule excludes other molecules from a volume of solution often much larger than its physical volume, particularly if it is extended rather than globular⁴. At sufficiently high concentration the freedom of polymer molecules to pack together, represented by an entropy term, becomes a significant factor favouring the segregation of polymer species of different molecular size. (For further discussion and thermodynamic treatment see refs 4 and 5.) So mixed solutions of hydrophilic polymers tend to show phase separation, and the larger the molecules the greater the tendency and the weaker the interactions needed to cause phase separation. It has already been pointed out that proteoglycans and glycoproteins ought to show this behaviour⁶.

If cells of different types have different glycoprotein coats they would in general be expected to form separate phases, that is, to sort out into aggregates of a single type of cell. If they are suspended in a medium such as plasma, containing small polymers, or even just saline, one phase might be cell-free, in other words cells of all types would aggregate, then sort out within the aggregate. (This, incidentally, may have something to do with the need for serum components in culture media for cells⁷.) It is well known that phase separation is a good description of the sorting out of embryonic cells. To quote Moyer and Steinberg⁸ "rearrangements [of cell aggregates] . . . resemble in striking detail the rearrangements of immiscible liquids", cells corresponding to molecules of the liquids.

The energy released by phase separation compares favourably with the energy needed to bind two cells together. Agglutination of two red cells requires less than 10^2 antibody molecules⁹ which would give about 10^3 kcal of binding energy per mol of cells. When, for example, two like cells come into contact over an area of $50 \mu\text{m}^2$, there is a loss of $100 \mu\text{m}^2$ of interface between the cells and either medium or unlike cells. For these cells to adhere as strongly as the red cells the interfacial free energy released by the contact would have to be only 10^3 kcal per $6 \times 10^{23} \times 100 \mu\text{m}^2$ or 7×10^{-5} erg cm^{-2} , much less than the 10^{-3} to 10^{-1} erg cm^{-2} typical of the examples of phase separation above³. It corresponds to only 2×10^{-3} kcal per mol of surface glycoprotein if the cell surfaces have the same surface density of integral membrane glycoproteins as the red cell¹⁰, 10^6 per $100 \mu\text{m}^2$.

So merely by carrying different glycoprotein coats cells might well be expected to show differential adhesion phenomena. For example, in development different cell types would form separate phases, that is, tissues. Less obvious examples could be the binding of desialylated serum glycoproteins to liver cell surfaces, though this may be mediated by a true lectin activity¹¹,

and the selective penetration of capillary walls by leucocytes, where the capillary wall can be viewed as a phase into which leucocytes can reversibly partition, but which is immiscible with other components of blood.

This explanation of apparently specific cell interactions in terms of large numbers of weak nonspecific forces conflicts particularly with the widely held view that cells interact through specific binding by recognition proteins with lock-and-key active sites^{1,2,11}. Such proteins probably do play a part in some mechanisms^{1,2,11}; the point is that they may not be necessary. Several authors¹ have previously suggested that the forces between cells are nonspecific; in particular, Steinberg¹² showed some years ago that nonspecific forces could produce immiscibility of aggregates of different types of cell. One way in which the consequences of the view presented here may differ from the consequences of the lock-and-key view is that immiscibility of dissimilar glycoprotein coats on different cell types can provide a repulsive interaction in the sense that active cell migration through an incompatible phase would be resisted. In contrast the simple lock-and-key picture tends to predict positive or zero adhesion.

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Hybrid cell lines secreting monoclonal antibody specific for major histocompatibility antigens of the mouse

HYBRIDISATION of a myeloma cell line with hyperimmunised spleen cells can provide hybrid cell lines secreting monoclonal antibodies of predefined specificity^{1,2}. Lines have thus been established which secrete homogeneous antibodies against antigens such as sheep red blood cells¹, the hapten 2,4,6-trinitrophenyl², influenza virus³, horse red blood cells, the haptens (4-hydroxy-3-nitrophenyl)acetyl and (4-hydroxy-5-iodo-3-nitrophenyl)acetyl, group A streptococcal carbohydrate, hen egg lysozyme, chicken γ globulin, the synthetic polypeptide poly-L-(Tyr, Glu)-poly-D,L-Ala-poly-L-Lys⁴ and antigens encoded by the major histocompatibility complex (MHC) of the rat⁵. We report here the derivation of three cloned hybrid cell lines which synthesise antibodies detecting different public antigenic specificities of the mouse H-2 complex. These hybrid cell lines can be grown in the mouse as ascites tumours, and the ascites fluids obtained contain large quantities of homogeneous anti-H-2 antibodies.

BALB/c (H-2^d) spleen cells hyperimmunised with CBA (H-2^k) lymphocytes were fused with the mouse myeloma P3-X63-Ag8 using polyethylene glycol as a fusion reagent (see

Fig. 1 legend). Culture supernatants of 200 individual hybrid cell lines obtained in one fusion experiment were screened for anti-H-2 activity by a complement dependent cytotoxicity assay or a cellular radioimmunoassay in which antibody bound to target cells was monitored by uptake of ¹²⁵I-labelled staphylococcal protein A (¹²⁵I-SpA)⁷. Five of the 200 supernates were cytotoxic to CBA but not BALB/c target cells. Their protein A assay response indicated that the secreted antibodies belonged to the IgG class⁸ (Fig. 1). No hybrid cell line was active in only one assay.

The hybrid cell lines were cloned in soft agar and three individual clones, one of each hybrid, with activity against CBA target spleen cells, were selected for further characterisation of their products. These clones, 5R4, 27R9 and 30R3, were injected intraperitoneally into Pristan-pretreated BALB/c mice. The hybrid cell antibodies were purified from ascites fluid by agarose block electrophoresis. Spectrophotometric determination of the protein concentration in different fractions revealed a single peak in the γ globulin region close to the starting point. The amount of purified protein in this peak was calculated from the optical density at 280 nm, assuming an extinction coefficient E (1 cm pathlength) of 1.4 for a solution containing 1 mg protein per ml. The yields were 40 mg from 4 ml ascites fluid for antibody 5R4, 45 mg from 3 ml ascites fluid for antibody 27R9, and 45 mg from 3 ml ascites fluid for antibody 30R3. Microzone electrophoresis and isoelectric focussing demonstrated that the protein peak contained both the P3-X63-Ag8 myeloma protein MOPC 21 (γ 1, κ) (refs 1, 12) and additional protein components, presumably representing the cytotoxic antibody and hybrid molecules¹². Most of the antibody activity was found in the fractions with the highest protein content. These were analysed in the Ouchterlony

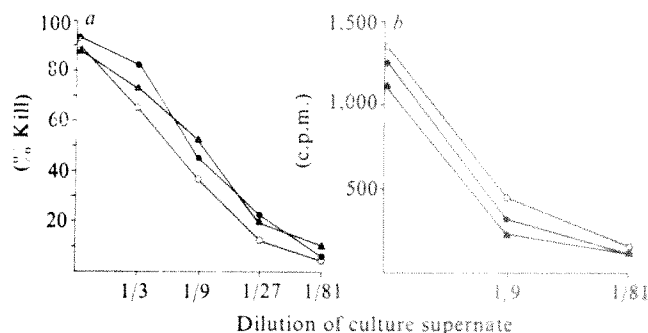


Fig. 1 BALB/c (H-2^d) mice were primed by skin grafting against CBA (H-2^k) allo-antigens and hyperimmunised by three intraperitoneal injections of $1-3 \times 10^7$ CBA spleen cells in 10-d intervals beginning with the first injection 2 weeks after priming. Five days after the last injection, cells from four BALB/c spleens were fused with the myeloma P3-X63-Ag8. Cells were fused employing a modification¹⁴ of the method described by Pontecorvo *et al.*¹⁵, except that polyethylene glycol with a molecular weight of 4,000 was employed and the ratio of spleen to myeloma cells was 2:1. After fusion, the cells were distributed in multiwell tissue culture plates (Costar No. 3524) at a density of 2×10^6 spleen cells per well and cultured in HAT medium¹. Two weeks later, cell growth became microscopically evident in 200 out of 240 wells. Culture supernatants of these positive wells were assayed for anti-H-2 activity either by a two step microcytotoxicity assay using rabbit serum as a source of complement (a gift from Dr J. Jensenius, Copenhagen) or by a cellular radioimmuno assay in which antibody bound to target cells is monitored by subsequent uptake of ¹²⁵I-labelled staphylococcal protein A which binds exclusively to immunoglobulin of the IgG class⁸. We followed the method described by Dorval *et al.*⁷. The titration curves for (a) cytotoxicity and (b) ¹²⁵I-binding properties of culture supernatants of hybrid cell lines 5 (●), 27 (○) and 30 (▲) are shown. Five out of 200 hybrid cell lines in this particular fusion experiment secreted anti-H-2 antibodies. One of the five hybrids lost its activity and another was lost by bacterial infection. In other fusion experiments, more than 1,000 hybrid cell lines were screened for anti-H-2 activity, but no positive lines were detected. At present we cannot define the precise experimental conditions to reproduce the successful hybridisation.

technique using class and subclass specific antisera (obtained from B. Liesegang & A. Radbruch, Cologne, and Meloy Co.). None of the three hybrid antibodies reacted with anti-μ and anti-α antisera. In all cases, precipitin lines appeared with anti-γ1 antisera, while antibody 30R3 reacted with anti-γ2b, and antibodies 5R4 and 27R9 with anti-γ2a. No reaction was found with anti-λ sera. This suggested that all preparations contain MOPC 21 chains as already indicated by microzone electrophoresis and isoelectric focussing, and that antibody 30R3 carries γ2b and antibodies 5R4 and 27R9 γ2a heavy chains. All three antibodies probably possess κ light chains.

Ascites fluids containing antibodies 5R4, 27R9 or 30R3 were tested on different H-2 haplotypes and intra-H-2 recombinant haplotypes to determine the antigenic specificities detected by these monoclonal antibodies (Table 1). The antibodies secreted by the three clones exhibited strong reactivity on CBA (H-2^k) but did not react with BALB/c (H-2^d) or DBA/2J (H-2^d) spleen cells. To determine whether H-2 specificities are detected by the antibodies, they were tested on B10.BR (H-2^k) and B10.D2 (H-2^d) target cells, which share the B10 background and differ only in their H-2 haplotypes. All three antibodies reacted with B10.BR (H-2^k) but not with B10.D2 (H-2^d), and thus can detect specificities of the H-2^k haplotype.

Various intra-H-2 recombinant strains were used to evaluate detection of the specificities of the K, I and D regions of the H-2 complex. All three antibodies reacted strongly with B10.A (H-2^a) target cells which have the K end of H-2^k and the D end of H-2^d. Antibody 5R4 did not react with C3H.OH (H-2^{o2}) cells which carry H-2^k antigens of the D region, while antibodies 27R9 and 30R3 occasionally showed weak reactions with these cells at high concentrations. Antibodies 5R4 and 27R9 did not react with the intra-H-2 recombinant A.TL (H-2^{tl}) or with A.SW (H-2^s), while antibody 30R3 showed reactivity with both strains. This indicates that antibodies 5R4 and 27R9 do not detect Ia antigens of the H-2^k haplotype and that the corresponding antigens are also not present in the K region of the H-2^s haplotype. It further suggests that antibody 30R3 also does not react with Ia antigens but crossreacts with a specificity of the K region of H-2^s. Another strong argument against the detection of Ia determinants by these antibodies is

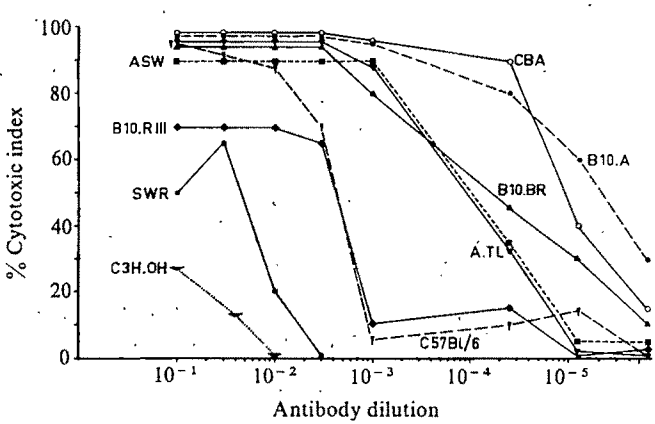


Fig. 2 Cytotoxic activity of antibody 30R3 on spleen target cells of different H-2 haplotypes. Ascites fluid of tumour-bearing BALB/c mice was used as the source of antibody 30R3. As the data were obtained in different assays, the cytotoxicity values are presented as cytotoxic indices (see legend to Table 1).

the fact that they kill close to 100% of spleen cells. This has not been observed with anti-Ia antisera since Ia antigens are predominantly expressed on B lymphocytes⁹. These data therefore suggest that all three antibodies detect determinants encoded by the K region of the H-2 complex.

Further experiments demonstrated that antibodies 5R4 and 27R9 showed no reactivity on C57BL/6 (H-2^b), while antibody 30R3 showed 100 to 1,000-fold lower cytotoxic activity than on CBA (H-2^k) or B10.BR (H-2^k) cells. All three antibodies reacted with antigenic specificities also present on B10.R.III cells which carry the H-2^r haplotype. Antibodies 5R4 and 30R3 but not 27R9 also displayed activity on SWR/J (H-2^s) cells. Comparison of these results with the distribution of antigenic specificities of the K region of the H-2 chart⁶ suggests that antibody 5R4 may detect specificity H-2.11, antibody 27R9 specificity H-2.25 and antibody 30R3 H-2.5. This was confirmed (Table 1) when all three antibodies were entirely negative when tested on A.CA (H-2^f) cells, a haplotype which expresses none of the H-2 specificities, 5, 11 and 25.

The differences in reactivities of antibodies 5R4 and 27R9 observed on target cells carrying different haplotypes may be due to experimental variations and do not necessarily reveal a polymorphism of the corresponding antigenic determinants. Antibody 30R3, however, reacted equally strongly with H-2^k and H-2^s cells, showed weak crossreactivity with haplotypes H-2^r and H-2^a and very weak reactions with H-2^{o2} target cells (Fig. 2). However, all these haplotypes express specificity H-2.5 encoded either by the K region (H-2^{b,k,s,r,q}) or by the D region (H-2^{o2}) of H-2 (ref. 6). It has already been suggested by conventional H-2 serology that specificity H-2.5 encoded by different H-2 haplotypes reflects non-identical but crossreactive antigenic determinants^{6,10,11}. Assuming that antibody 30R3 really detects specificity H-2.5, the titre differences observed in the reactions of this antibody with target cells from different haplotypes (Table 1, Fig. 2) seem larger than titre differences with conventional anti-H-2 antisera, which have relatively low titres. This may be due to the different strain combinations used for raising conventional antisera⁶ and for the production of antibody 30R3. In addition, even if the same strain combination had been used for the production of the hybrid cell antibody, one might still expect the fine specificity of this monoclonal antibody to be different from that of a complex antiserum. It is indeed striking to see that the antigenic specificities detected by our three hybrid cell antibodies correlate largely with antigenic specificities enlisted in the H-2 chart⁶. The exquisite specificity of serological typing reaction thus seems to be fully retained at the level of monoclonal antibodies. This finding is important¹³, and suggests that monoclonal antibodies will play a key role in future histocompatibility typing.

We thank Dr C Milstein for the gift of the myeloma P3-

Table 1 Cytotoxicity of BALB/c anti-CBA (H-2^d anti-H-2^k) monoclonal antibodies obtained from ascites fluids of tumour bearing mice

Strain	H-2 Haplotype	H-2 Complex			Cytotoxic titre of antibody*		
		K	I	D	No. 5R4	No. 27R9	No. 30R3
CBA	k	k	k	k	90,000	40,000	100,000
BALB/c	d	d	d	d	—	—	—
DBA/2	d	d	d	d	—	—	—
B10.BR	k	k	k	k	25,000	20,000	20,000
B10.D2	d	d	d	d	—	—	—
B10.A	a	k	k/d	d	25,000	20,000	200,000
C3H.OH	o2	d	d	k	—	†	†
A.TL	tl	s	k	d	—	—	9,000
C57BL/6	b	b	b	b	—	—	400
A.SW	s	s	s	s	—	—	10,000
B10.R.III	r	r	r	r	25,000	100,000	400
SWR	q	q	q	q	4,000	—	50
A.CA	f	f	f	f	—	—	—

*Reciprocal antibody dilutions at which 50% killing of target spleen cells of different H-2 haplotypes were obtained. The data are corrected for background killing as follows:

cytotoxic index (%) =
$$\frac{\% \text{ dead cells with antisera} - \% \text{ dead cells control}}{100 - \% \text{ dead cells control}} \times 100$$

The 50% values are taken from complete titration curves an example of which is presented in Fig. 2.

†In some tests cytotoxic activity was observed at antibody concentrations ≥ 10.

X63-Ag8. This work was supported by the Deutsche Forschungsgemeinschaft.

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Cytotoxic T cells learn specificity for self H-2 during differentiation in the thymus

CELL mediated immune reactions in mice involving cytotoxic thymus derived lymphocytes have two important features; they are antigen-specific¹ and the structures coded for by the major murine histocompatibility gene complex (H-2) play an important part in killer cell-target cell interaction¹. This finding is not unique to mice since it has also been reported for rats, humans and chickens²⁻⁴. Two hypotheses have been suggested to explain this dual specificity of T killer cells; one proposes that T killer cells possess two separate structures, one of which recognises self H-2 structures and the other a non-H-2 antigen (dual recognition). The other hypothesis proposes that T killer cells possess one receptor which recognises an H-2 antigen modified by a non-H-2 antigen (modified self)^{1,5}. Evidence to test these hypotheses was sought by preparing chimaeras made by reconstituting lethally irradiated F₁ mice with bone marrow stem cells of one parent (Parent→F) refs 6-8 or neonatally tolerant mice⁹ (mice of a parental H-2 type injected with F₁ cells as neonates). As a result, Parent → F₁ chimaeric lymphocytes lysed

infected and trinitrophenyl (TNP)-modified targets of both parental H-2 types, suggesting that H-2 antigens of the killer cells were not involved in the lytic interaction. In contrast, lymphocytes from neonatally tolerant mice did not lyse infected targets from the incompatible parent⁹. Although these results can be explained by postulating a single receptor, they also fit the dual recognition hypothesis⁶⁻¹⁰. Bevan has shown that irradiated bone marrow chimaeras of the type (A × B)F₁ → Parental A recipient generated preferentially cytotoxic T cells against minor histocompatibility antigens in association with the H-2 haplotype of the recipient A; he concluded that the chimaeric host determines the specificity of T cell restriction either through a thymic selection process as proposed by Jerne or alternatively through an antigen presentation mechanism.

In similar experiments with F₁ → Parent chimaeras, we found that tolerance alone was again not sufficient to generate both virus-specific and TNP-specific cytotoxic T cells against antigen in association with the tolerated H-2. These results and studies on the role of the thymus in the differentiation of H-2 restricted killer cells seem to indicate that precursor T cells from lymphohaemopoietic stem cells are selected to recognise the H-2 markers during their differentiation in the thymus; this learning process is independent of the T cell's own H-2 haplotype¹⁶.

F₁ hybrid → Parent chimaeras were formed by irradiating parental mice with 925-950 rad and then reconstituting them with F₁ bone marrow cells⁷. The chimaeras were infected 10-12 weeks later with vaccinia virus [10⁷ plaque forming units (PFU) per recipient] and killed 6 d later. Spleen cells from each individual were typed serologically for H-2, after which virus-specific cytotoxicity was assessed in a ⁵¹Cr release assay on infected and uninfected target cells (Table 1). Spleen cells from other F₁ → Parent chimaeras were also typed for H-2 and then sensitised *in vitro* against TNP-modified stimulator cells (Table 2). The serological results show that the lymphocytes of the F₁ → Parent chimaeras were all of F₁ type. However, cytotoxic activity was directed exclusively against infected or TNP-modified targets of the recipient parental type but not against normal targets of both parental H-2 types either *in vitro* or *in vivo*. Thus, cytotoxicity was found only against targets that were H-2 identical with F₁ donor and parental chimaera recipient, but not against targets of the second parental H-2 type, despite the inherent tolerance of F₁ lymphocytes for both parental antigens and the presence of large amounts of infected or TNP modified cells expressing all relevant H-2 antigens. Other chimaeras were constructed of the type (A × B)F₁ → (A × C)F₁. When lymphocytes from such chimaeras were tested in the TNP system and sensitised against TNP (B × C)F₁ cells, strong TNP-specific cytotoxicity was generated against C-TNP but none against B-

Table 1 Vaccinia-virus specific cytotoxic activity generated in F₁ → Parent irradiation bone marrow chimaeras *in vivo*

Immune lymphocytes from vaccinia infected mice* (H-2 type)	Lymphocyte to target cell ratio	% Specific ⁵¹ Cr release from target cells			
		L(k)		D2.GD(d/b)	
		Vaccinia treated	Normal	Vaccinia treated	Normal
Bone marrow chimaeras					
BALB/c × A → A	40:1	81††	0	5	2
(d × k/d) (k/d)	13:1	45††	2	2	—
BALB/c × A → BALB/c	40:1	3	2	51†	1
(d × k/d) (d)	13:1	5	3	35†	2
Normal mice					
BALB/c	40:1	2	0	60†	2
(d)	13:1	—	1	22†	5
A	40:1	98†	2	—	—
(k/d)	13:1	80†	1	—	—
C3H	40:1	97†	3	4	1
(k)					

* Recipient mice were irradiated with 925 rads and reconstituted with 2 × 10⁷ twice anti-θ plus C treated bone marrow cells. Chimaeras were infected 10 weeks later with about 10⁷ plaque forming units of vaccinia virus WR⁷. Their spleen cells were typed for H-2; for both chimaeras > 95% of the lymphocytes were lysed by anti-K^b and anti-K^d antiserum. Virus specific cytotoxic T cells were tested for 6 h on infected or uninfected target cells⁹. The spontaneous release from L cells was about 25%, from D2.GD macrophages about 45%.

† The results are given as means of triplicate determinations that were corrected for spontaneous release; water released about 80% of the incorporated ⁵¹Cr and this was taken as 100% release; s.e.m. were smaller than 5%.

‡ Significantly different from relevant controls (P < 0.01).

Table 2 TNP-specific cytotoxic activity generated by $F_1 \rightarrow$ Parent or $F_1 \rightarrow F_1$ chimaeric lymphocytes *in vitro*

Responder* lymphocytes (H-2 type)	Stimulator lymphocytes	Target cells†	% Specific ^{51}Cr release from targets‡	
			TNP	Normal
Chimaeric mouse BALB/c \times A \rightarrow A (d \times k d) (k d)	TNP-BALB/c \times A (d \times k d)	C3H (k) D2.GD (d b)	62 <1	<1 <1
Normal mouse BALB/c (d)	TNP-BALB/c (d)	BALB/c (d) D2.GD (d b)	93 28	47 .18
Chimaeric mouse BALB/c \times \rightarrow C3H \times C57BL/6 BALB/c (d \times b) (k \times d)	TNP-C3H \times C57BL/6 (k \times b)	C3H (k) C57BL/6 (b)	65 <1	8 <1
Normal mice C3H (k) C57BL/6 (b)	TNP-C3H (k) TNP-C57BL/6 (b)	C3H (k) C57BL/6 (b)	61 90	<1 <1

*Chimaeras were prepared and typed for H-2 as in Table 1. Spleen cells (10^7 ml^{-1}) were cultured for 5 d with trinitrobenzene sulfonate (TNBS) coupled stimulator spleen cells ($5 \times 10^5 \text{ ml}^{-1}$) irradiated with 1,000 rad as described previously¹².

†Spleen cells were (10^6 ml^{-1}) cultured for 3 d in the presence of $10 \mu\text{g ml}^{-1}$ bacterial lipopolysaccharide, collected and coupled with TNBS and labelled with Cr^{51} (ref. 12). The attacker to target cell ratio was 50:1.

‡Values are means of duplicates corrected for spontaneous release. The 100% release was determined by freezing and thawing the cells 3 times. s.e.m. values were smaller than 5%. The 4-h time point is given.

TNP (Table 2). This indicates that stem cells differentiate in the irradiated recipient to acquire H-2 (C)-restricted specificity that is host-dependent but independent of the H-2 type which the lymphocytes express themselves. The results from neonatally tolerant mice can be similarly explained⁹.

These results are compatible with the general idea of 'adaptive differentiation'¹⁰ and agree with similar data of Bevan¹¹, apparently implying that the H-2 type of the host in which T lymphocytes differentiate governs the specificity of subsequent H-2 restriction. One would therefore not expect parental strain cells depleted of alloreactivity to lyse antigen-modified fully allogeneic cells. Schmitt-Verhulst and Shearer¹³ showed that

lymphocytes of A origin which were deprived of anti B-alloreactivity by bromodeoxyuridine (BUdR) and light could not be sensitised specifically against B-TNP. However, their data and ours disagree with those of Wilson *et al.*¹⁴, who found that lymphocytes of A origin that were deprived of anti-B-alloreactive cells by filtration through irradiated F_1 animals could be sensitised specifically against B-TNP. This discrepancy cannot yet be resolved, but the different reactivities may have arisen from incomplete tolerance of the filtered lymphocytes or differences in the method of TNP modification.

Our results indicate that the chimaeric host determines the H-2 restricted specificity of cytotoxic T cells; they illustrate clearly

Table 3 Virus specific cytotoxic T cell activity generated in adult thymectomised irradiated C57BL/6 mice that were reconstituted with anti- θ treated F_1 bone marrow cells and transplanted with irradiated F_1 thymus of BALB/c \times C57BL/6 origin.

Bone marrow donor	Thymus donor	Irradiated recipient	Lymphocyte to target ratio	% Specific ^{51}Cr release from target cells			
				Vaccinia treated	MC57G(b) Normal	Vaccinia treated D2(d)	Normal
BALB/c \times C57BL/6 (d \times b)	BALB/c \times C57BL/6 (d \times b)	C57BL/6 (b)					
Thymus tissue detectable in kidney			40:1 13:1 4:1	35* 14† 1	2 4 1	60† 21† 9	5 3 6
Thymus tissue not detectable in kidney			40:1 13:1	3 2	1 2	4 2	5 2
Vaccinia virus infected control mice C57BL/6 (b)			40:1 13:1	95† 45†	6 2	6 2	4 2
BALB/c (d)			40:1 13:1	5 2	4 2	100† 55†	2 3

Recipient mice were thymectomised, 1 week later irradiated (950 rads) and reconstituted with twice anti- θ plus C treated bone marrow cells of F_1 origin; irradiated (900 rads) thymus lobes from 6-8-week-old F_1 donors were transplanted under the kidney capsule. These thymus chimaeras were infected about 3 months after reconstitution. 6 d later the animals were killed, the spleen cells typed for H-2 ($> 95\%$ were positive for H-2^b and H-2^d) and tested for cytotoxic activity in a 16-h test. Absence of the thymus of the host and presence or absence of thymic tissue under the kidney capsule were controlled histologically.

*Means of triplicate determinations; values are corrected for spontaneous release (MC57G: 26%, D2: 40%) and water release was taken as 100%.

†Significantly different from controls ($P < 0.05$).

that peripheral tolerance to H-2 is not sufficient to allow recognition by T cells of this same H-2 together with antigen. Thymus transplantation experiments indicate that it is the H-2 type of the thymus epithelial cells which determines the H-2 antigens treated as self by H-2 restricted T cells (Table 3). Adult thymectomised, irradiated C57BL/6 mice were reconstituted with bone marrow of BALB/c \times C57BL/6 origin and transplanted with two irradiated thymus lobes of BALB/c \times C57BL/6 origin under one kidney capsule. When infected with vaccinia virus 3 months later, cytotoxicity was generated for both parental H-2 type infected targets. This is in contrast with the results from $F_1 \rightarrow$ Parent irradiation bone marrow chimaeras, where activity was formed for the compatible recipient parental H-2 type only.

These two models differ with respect to the thymus of the two chimaeras and we may therefore conclude that the radioresistant part of the thymus, probably the H-2 specificity of the thymic epithelium, determines what differentiating T cells learn to recognise as self-H-2. Since thymectomy was histologically complete, and since virus-specific cytotoxic activity was generated for both infected H-2^b and H-2^d targets, and not for H-2^b alone, it follows that the transplanted thymus induced T cell maturation. The anti- θ plus C treatment was complete in depleting T cells, otherwise one would have expected that mature contaminating F_1 T cells would have given rise to a significant level of cytotoxicity in the second chimaera from the same group of animals, where no histological or functional thymus reconstitution was achieved. Therefore, it seems that precursor T cells learn to recognise as self the H-2 antigens expressed by the radioinsensitive portion of the donor thymus, that is, thymus epithelium. This capacity for self recognition differentiates independently from the H-2 haplotype of precursor T cells and independently from recognition of non-H-2 antigens.

Can these results best be explained by the single or dual recognition model? Lymphocytes from F_1 (A \times B) \rightarrow P(A) chimaeras generate antigen-specific killer T cells against H-2-compatible targets of parent A only, despite the fact that they were exposed to antigen in association with both A and B parental H-2 antigens. This could be explained by Jerne's speculation¹⁵ that T cells of a mouse A evolve from proliferating and mutating T cells that were specific for A to express specificity for 'different from A'. Within such a concept the one receptor hypothesis, which suggests that H-2 restriction results from an association of non-H-2 antigen at the level of induction could explain this experimental finding only if the rule were made that the specificity spectrum for modified self generated in thymus A cannot overlap with that generated in thymus B. This rule is unpredicted and unlikely, but cannot be excluded.

Because there is no precedent for the H-2 haplotype of the thymus restricting T cell-specificity (except perhaps for *Ir* gene effects), the explanation that T cells have independent receptor sites for thymus epithelial H-2 and for non-self antigen, and operate through dual recognition may be the more attractive one. Whether the two recognition structures are on one or two membrane molecules is not clear. These results and the concept that self-specificity is selected for in the thymus have important theoretical and practical implications for further experimentation. They may also provide an improved rationale for clinical attempts at reconstitution of immunodeficiency in humans. If the presented results are correct and apply to humans, it is predicted that (except for pure thymus hormone deficits) reconstitution of immunodeficient patients with completely HLA incompatible thymus epithelium or lymphohaemopoietic stem cells may result in differentiation of mature T cells that cannot interact with other T cells or B cells because they were taught in the thymus to recognise HLA structures as self that are not of their own HLA type. Therefore, functional immunological reconstitution of thymus and/or stem cell deficient patients with immunoincompetent thymus tissue or liver cells, for example, of foetal origin (to avoid graft versus host reactions), may depend on recipient and donor sharing at least one HLA-A or B and D haplotype.

We thank Drs D. H. Katz and B. S. Skidmore for anti- θ serum, Alana Althage and Sue Cooper for technical assistance and

Elizabeth Sinclair and Phyllis Minick for secretarial help. This work was supported by USPHS grants A1 07007, A1 13779, CA 19108, A1 11650, CA 15581 and CA 19334.

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Received 26 October; accepted 7 December 1977.

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Cell surface changes in alloantigen activated T lymphocytes

MANY interactions which take place between T lymphocytes and other cells are presumably mediated at the level of the cell surface. In a mixed leukocyte culture (MLC), interaction between allogeneic lymphocytes results in transformation of the responding small T lymphocytes to large blast-like cells and their development into killer, helper¹ and suppressor lymphocytes². Some blasts subsequently revert to small memory or 'secondary-type' lymphocytes³. An important question is whether significant changes occur in T lymphocyte membrane proteins as a result of these interactions. We have attempted to answer this question by comparing iodine-labelled surface proteins of the following cells: T lymphocytes before stimulation, blast cells and small lymphocytes purified by Ig velocity sedimentation from an MLC on day five, and reversion T lymphocytes following an additional 5 d of culture of the separated blast T lymphocytes. The results demonstrate that iodine-labelled components change during blast transformation and provide evidence for membrane differences between 'secondary-type' T lymphocytes and unsensitised cells.

Figure 1 shows radioactivity profiles resulting from co-electrophoresis of NP-40 extracts of ¹²⁵I-labelled murine thymus and spleen and ¹³¹I-labelled nylon column purified spleen T cells on a 5% acrylamide, 0.1% sodium dodecyl sulphate (SDS) gel. When analysed on these 5% gels, both spleen and nylon column purified T cells, Fig. 1b show three distinct peaks of radiolabelled proteins which have molecular weights of approximately 200,000 (200K), 187K, and 170K. (Preliminary SDS gel studies of plasma membrane preparations (G. Sundharadas, to be published) from ¹²⁵I-labelled cells also show three peaks in the 170K to 200K molecular weight

range). The presence of these peaks in the thymus preparation (Fig. 1a) and in nylon wool purified cells and the absence of any of the three peaks in macrophage or nude mouse spleen cell preparations (data not shown), are consistent with these being specific T cell-associated peaks. Trowbridge *et al.*⁴, using a 7.5–15% gradient gel, have reported the presence of a T cell-specific protein of molecular weight 200K in preparations similar to ours. The peak of molecular weight 240K in Fig. 1b

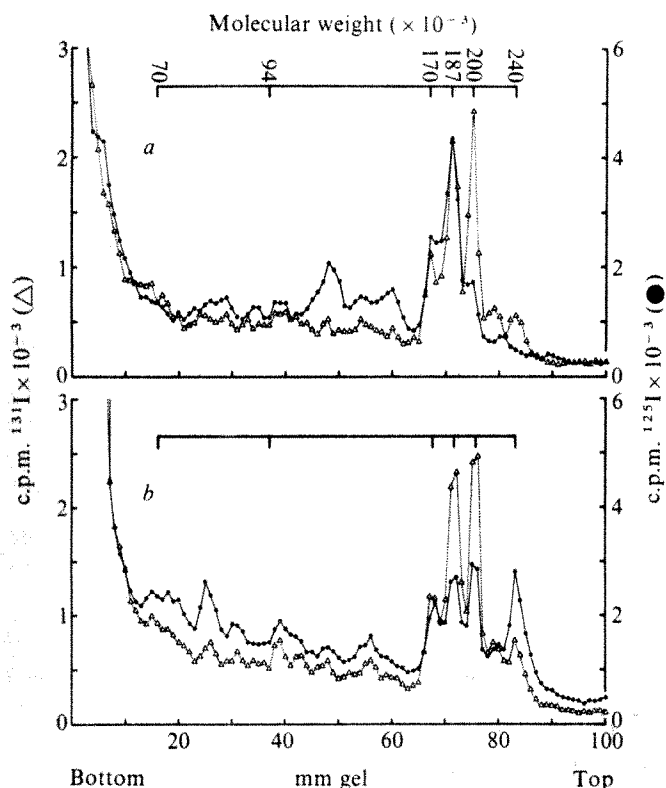


Fig. 1 NP-40 extracts of ^{125}I -labelled murine spleen and thymus and ^{131}I -labelled nylon column purified cells electrophoresed on SDS-polyacrylamide gels. Single cell suspensions were prepared by aseptically removing the spleen or thymus, and teasing them through a wire mesh into EHAA media. Aggregates were allowed to settle, the cells aspirated off and washed once in media. Erythrocytes were removed from the spleen cell suspensions by lysing with 0.15M NH_4Cl , 0.01M Tris Cl, pH 7.2. Nylon column purified T cells were prepared from spleen cell suspensions by passage over a nylon wool column according to Julius *et al.*⁷. Cells were washed three times in PBS, then $2\text{--}4 \times 10^7$ cells in 0.5 ml were iodinated with 1 mCi ^{125}I or ^{131}I (total concentration of iodide was $1 \times 10^{-6}\text{M}$) using the galactose-oxidase lactoperoxidase technique of Schenkein *et al.*⁸. Cell viability was determined before and after iodination; cell recovery was $>95\%$ and $>90\%$ of the cells were viable (by eosin staining) before and after iodination. After iodination, cells were washed three times with PBS, and lysed by addition of Nonidet P40 (Shell) to a final concentration of 0.2%. Nuclei were removed by centrifugation at 1,000g for 10 min. The supernatants were made 3% in SDS, 0.1M in β -mercaptoethanol, heated to 90°C for 3 min, and indicated samples co-electrophoresed on 6 mm \times 110 mm, 5% acrylamide 13% bi-sacrylamide gels containing 0.1% SDS, according to Laemmli⁹. Electrophoresis was stopped when the tracking dye reached the lower edge of the gel, and the gels separated into 1 mm fractions using a Gilson gel fractionator model B-200 GMA-GCB. Scintillator fluid (3a70B, Research Products International, Incorp.) 3 ml, was added to each fraction and the fractions counted in a Packard tricar liquid scintillation spectrometer model 3330 using window settings of 50–1,000 (^{125}I), 200–1,000 (^{131}I) and a gain of 50% (^{125}I) or 6% (^{131}I). The net counts of ^{125}I were corrected for overlap of ^{131}I counts into the ^{125}I -channel. Approximate molecular weights of the peaks were calculated from their position relative to those of known standards. *a*, Extracts of ^{125}I -labelled thymus cells (\bullet) and ^{131}I -nylon wool cell extracts (Δ). *b*, Extracts of ^{125}I -labelled spleen cells (\bullet) and ^{131}I -nylon wool cell extracts (Δ).

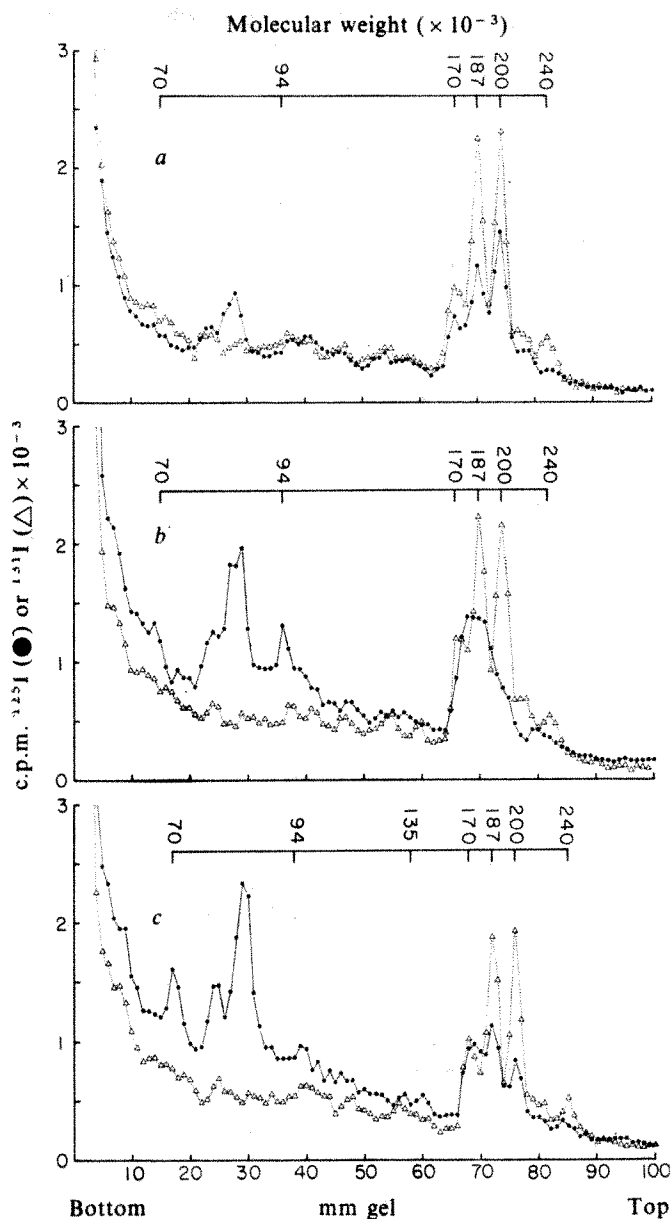


Fig. 2 NP-40 extracts of ^{125}I -labelled small lymphocytes, blasts, reverted cells and ^{131}I -nylon wool purified T cells, electrophoresed on SDS-polyacrylamide gels. Mixed leukocyte cultures were set up in 250 ml Falcon tissue culture flasks (50–75 ml media per flask), using culture conditions according to Peck and Bach¹⁰. Nylon wool purified T cells from B10.M mice were prepared as described in Fig. 1, and stimulated with allogeneic (B10.G) γ -irradiated (2,000R) spleen cells, using 2.5×10^6 responding and stimulating cells per ml. After 5 d in culture, cells were harvested, washed with PBS and separated into large, rapidly sedimenting 'blast' cells and small, slowly sedimenting lymphocytes on a 5–30% horse serum gradient by unit gravity velocity sedimentation¹¹. Cells were allowed to settle for 4 h, the gradient fractions checked microscopically, and fractions containing either pure blasts or pure small lymphocytes were pooled. Samples of each were washed three times in PBS, labelled with ^{125}I and extracted with Nonidet P-40, as described in Fig. 1. The remaining blasts were then cultured for 5 d in 50 ml Falcon tissue culture flasks, containing 10^7 cells in 10 ml EHAA medium, at 37°C in a 5% CO_2 atm, after which they were collected, washed three times, in PBS labelled with ^{125}I and extracts prepared as in Fig. 1. No blasts were evident in these 'reverted' cell cultures. The ^{125}I -labelled extracts were co-electrophoresed with ^{131}I -labelled extracts from nylon column purified T cells, as described in Fig. 1. *a*, Extracts of ^{125}I -labelled small lymphocytes from 1g gradient (\bullet) and ^{131}I -nylon wool cell extracts (Δ). *b*, Extracts of ^{125}I -labelled blast cells from 1g gradient (\bullet) and ^{131}I -nylon wool cell extracts (Δ). *c*, Extracts of ^{125}I -labelled reverted cells (\bullet) and ^{131}I -nylon wool cell extracts (Δ).

which is present in the spleen cell preparation is probably a B cell-associated protein, as it is greatly reduced in extracts made from cells passed through nylon wool columns (Fig. 1b), and is absent in macrophage and blast cell extracts.

Figure 2a and b shows the profiles of nylon separated lymphocytes compared with extracts of labelled small lymphocytes and blast cells, respectively, after separation on a 1g gradient from a 5-d-old MLC. Blast cells activated by allogeneic cells (Fig. 2b) have the three distinct peaks of molecular weights 200K, 187K and 170K, replaced by a broad band of radiolabelled proteins covering the molecular weight range of 170–200K. In addition, a new peak or greatly increased component of 94K has appeared and several changes in the intensity of other peaks have occurred. The small lymphocytes from the 1g gradient retain the three distinct peaks (200, 187 and 170K) present in the starting nylon column purified cells, as well as most of the other starting cell peaks.

In contrast to the blast cells, reverted cells (Fig. 2c) have three discernible peaks of radioactivity in the 170–200K molecular weight range. The 200K and 187K peaks are the same as the peaks in the nylon column purified cells, but the third peak (170K in the nylon column cells) is reproducibly broader and shifted to slightly higher molecular weight. There is an increase in the amount of the 200K peak in reverted cells compared to blast cells. In addition, the 94K peak in the blast cells is greatly reduced or missing from the reverted cells, while a peak of 70K is prominent in the reverted cells but not in the blast cells. In comparison to the starting nylon wool cells, the reverted cells have different proportions of the 170K, 187K and 200K peaks, and peaks of 70K and 90K have appeared which are absent or present in low amounts in the starting cells.

The experiments presented demonstrate that T cells obtained from the blast cell fraction of a 1g gradient that have undergone functional change after allogeneic stimulation have different cell surface ^{125}I -labelling patterns from those of a starting population of T cells obtained from nylon wool or small lymphocytes obtained from the 1g gradient. The differences are consistent with the surface differentiative changes which take place as a small lymphocyte develops into a blast cell. These changes, however, may be associated with the cell cycle or represent alterations in the accessibility to iodination of common components due to changes in other components. They may also reflect expression of new blast-specific surface structures or modification of molecules existing in the non-stimulated cell, for example, by glycosylation. Also, selection of a clonal type, where cells of different responding clones give rise to functionally similar blasts which have different surface patterns, can be ruled out because we have observed the same changes where stimulating cells from different strains were used (unpublished data). Further, one might not expect variation in the molecular weight (as measured in SDS) of a given membrane product in different clones of cells with the same function.

More difficult to evaluate is whether these changes could reflect selection, during culture, of one or more functionally distinct T cell subpopulations with the observed ^{125}I -banding patterns. We regard this as unlikely in view of our current understanding that the T cell subpopulations present and responding in the initial T cell population^{5,6} are the same as and occur in similar proportion (A. Senik & B. R. Bloom, personal communication) to those found in the blast fraction. The interpretation of our results as a selection process could be clarified if the starting population could be more specifically defined, by raising antisera to the proteins that differentiate the starting small lymphocytes, the blasts and reversion lymphocytes and using these sera to dissect the various populations.

The blast T lymphocyte population analysed on these gels consists of several functionally different T cell populations. The majority of T lymphocytes present in the blast cell fraction are probably proliferating helper T cells, while a small percentage are cytotoxic T lymphocytes, and some suppressor T lymphocytes may be present. We are undertaking further experiments to determine if these functionally different blast

cell populations also differ in their cell surface components.

This work is supported in part by NIH grants CA-16836, AI-11576, AI-08439 and National Foundation-March of Dimes grants CRBS 246 and 6-76-213.

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Received 22 April; accepted 17 November 1977.

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Cyclophosphamide inhibited B cell receptor regeneration as a basis for drug-induced tolerance

A WIDE range of immunosuppressive agents can facilitate the induction of specific tolerance by thymus-dependent antigens. In the case of cyclophosphamide (CY), this involves T cells (including the generation of suppressor T cells), whereas B cells remain relatively unaffected^{1–3}. On the other hand, CY lowers the B cell tolerance threshold to thymus-independent (TI) antigens^{4,5}, a characteristic not shared by other alkylating agents (melphalan and chlorambucil) or immunosuppressive drugs (6-mercaptopurine, azathioprine and A-methopterin) (J. G. Howard and C. Hale, in preparation). Although high doses of CY induce selective depletion of B cells^{6,7}, its immunosuppressive potential *in vivo* or *in vitro* is manifest with doses well below this cytotoxic threshold (F. L. Shand, in preparation), which is not the case with melphalan and chlorambucil. Consequently, the B cell tolerance-promoting and reversible immunosuppressive activities of CY might be dissociable from its cytotoxicity. We have investigated the ability of splenic B lymphocytes from CY-injected mice to cap and subsequently regenerate their surface immunoglobulin (SIg) receptors following treatment with anti-immunoglobulin. Re-expression of B cell SIg was grossly impaired following treatment with CY, but not with other immunosuppressive agents. This finding provides a possible explanation for the increased susceptibility of B cells to tolerance induction with TI antigens.

Mice of both sexes from various strains (BALB/c, CBA/Lac, CBA/H and CBAx57BL)F₁ were used between 3 and 6 months of age. Cyclophosphamide monohydrate (Koch-Light), melphalan, chlorambucil and 6-mercaptopurine (Wellcome) were injected intraperitoneally at 150, 10, 30 and 75 mg per kg, respectively. Melphalan and chlorambucil were used at their maximally tolerated doses; in the case of CY, this exceeds 300 mg per kg. Spleen cell suspensions were treated with rhodamine-labelled rabbit-anti-mouse Ig (supplied by Dr L. Hudson) for 45 min at 37 °C. After three washes, 1×10^7 cells were cultured in Marbrook chambers and samples collected periodically for indirect immunofluorescent staining in 0.13% sodium azide using the (Fab')₂ fragment of rabbit-anti-mouse Ig (pre-

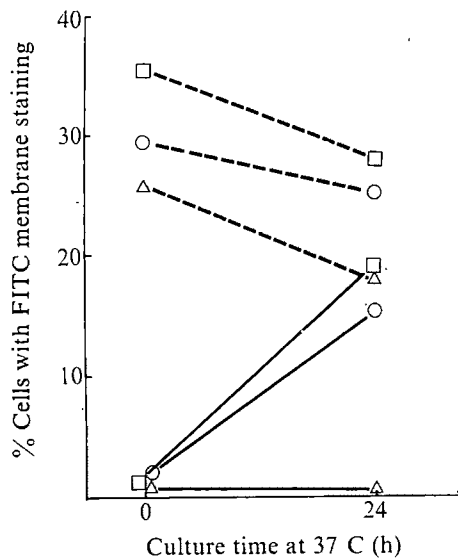


Fig. 1 Comparison of SIg receptor regeneration of spleen cells from normal (□) melphalan-injected (10 mg per kg) (○) or CY-injected (150 mg per kg) (△) mice, after treatment with anti-Ig serum. Spleen cells (2.5×10^7) were incubated in 0.5 ml of rhodamine-labelled rabbit-anti-mouse Ig serum (1/40) for 45 min at 37 °C. Suspensions were washed three times and 1×10^7 cells cultured in RPMI + 5% foetal calf serum in Marbrook chambers for 24 h at 37 °C in 5% CO₂. Cells were collected and stained in 0.13% sodium azide with an indirect immunofluorescence assay using a (Fab')₂ fragment of rabbit-anti-mouse Ig (1/20) followed by a FITC-labelled sheep-anti-rabbit Ig (1/20). The percentage of total lymphocytes expressing new FITC-labelled SIg was based on counts of 200–300 cells. Dashed lines represent the levels of SIg-bearing cells in control preparations which were not treated with rhodamine-labelled anti-Ig serum.

pared by pepsin digestion and fractionation on ultragel Ac-44) followed by fluorescein-labelled sheep-anti-rabbit Ig (Wellcome Reagents Ltd). This protocol enabled us to monitor both the frequency of capping and the reappearance of new SIg. The use of a (Fab')₂ fragment excluded the possibility of detecting Fc receptors. In enumerating those B cells which expressed new SIg, we recorded as positive only those cells which showed typical ring membrane fluorescence with fluorescein in the absence of rhodamine. Whereas normal spleen suspensions after anti-Ig treatment had levels of B cells expressing new SIg which were 40 and 75% of control values at 6 and 24 h respectively, a corresponding increase in B cells expressing new SIg was not detected in spleen suspensions from mice injected with

Table 1 Percentage of total lymphocytes expressing new SIg at various times after anti-Ig treatment

% Cells showing membrane staining with FITC-anti-Ig only at (h)	Normal mice		Mice injected with 150 mg per kg CY 20 h previously	
	RAM-Rh*	Medium only	RAM-Rh	Medium only
0	<1.0	37.9	<1.0	22.8
6	14.6	35.1	2.8	23.3
24	25.2	33.3	1.0	16.6
% caps immediately after	91	—	93	—
RAM-Rh treatment				
% viability at 24 h†	55	58	45	49

*RAM-Rh, spleen cells were incubated in rhodamine-labelled rabbit-anti-mouse Ig for 45 min at 37 °C and washed three times.
†Determined by Trypan blue dye exclusion. Other details as in Fig. 1.

CY 20 h earlier (Table 1). This result was obtained consistently in four experiments. Although a 30% reduction in the number of B cells recovered from CY-injected mice was always found, the frequencies of cells capping were essentially similar in either type of suspension. The percentage viability of cells from CY-injected mice was only marginally reduced after 24 h of culture as compared with normal cells. Analogous impairment of SIg receptor regeneration has also been reproduced with normal spleen cells previously treated with CY activated by liver microsomes *in vitro* (F. L. Shard, in preparation). In other experiments, spleen cells from CY-treated mice were compared with those from normal or melphalan-treated mice. Figure 1 shows typical impairment of SIg regeneration in CY- but not melphalan-injected mice. In another experiment, neither chlorambucil nor 6-mercaptopurine caused any impairment of SIg receptor regeneration. Thus, the failure of B cells from CY-injected mice to regenerate their SIg receptors is a distinctive sequel to treatment with this drug not shared by the other immunosuppressive agents. To ascertain whether this abnormal behaviour of B cells 20 h after CY injection was a reversible phenomenon, spleen cells from mice injected with CY 1, 3 or 7 d previously were examined for their ability to regenerate SIg after anti-Ig treatment. Partial recovery of SIg regeneration was found 3 d after CY and full recovery by 7 d (Fig. 2a). The immune status of parallel groups of mice was established by challenge with either levan or sheep red blood cells at the same time intervals after CY treatment. The levels of SIg recovery correlated with the extent of immune response following challenge with both T-dependent and T-independent antigens at the same times (Fig. 2b). In a final variant of these experiments, the incubation

Fig. 2 a, Comparison of SIg receptor regeneration of spleen cells obtained from mice injected 1, 3 or 7 d previously with 150 mg per kg CY after treatment with anti-Ig serum. Percentage of cells showing typical membrane ring fluorescence; before anti-Ig treatment (open columns), after anti-Ig treatment and 24 h of culture (shaded columns) and without anti-Ig treatment after 24 h of culture (stippled columns). Other details as in Fig. 1. **b**, Plaque-forming cell (PFC) response to sheep red blood cells (SRBC) (open columns) and levan (shaded columns) in groups of mice injected 1, 3 or 7 d previously with 150 mg per kg CY ($n = 5$). Mice were immunised with 5×10^8 SRBC or 10 µg levan i.v. and direct PFC assay performed 5 d later. SRBC were conjugated with levan as described by Miranda¹³.

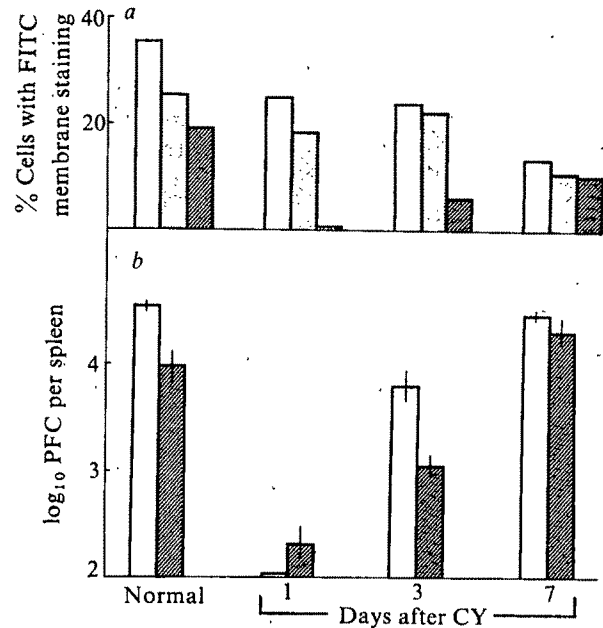


Table 2 The inability of CY-treated spleen B cells to regenerate their SIg is irreversible

% Cells showing membrane staining with FITC-anti-Ig only at (h)	Spleen cells from			
	Normal mice	Mice injected with 150 mg/kg CY 24 h previously	RAM-Rh*	Medium only
0	<1.0	40.1	<1.0	31.2
24	24.6(79)	38.5(85)	1.7(63)	29.5(73)
48	18.9(53)	32.7(65)	1.2(44)	18.1(49)
72	16.3(27)	21.8(30)	1.0(25)	13.5(26)

Figures in parentheses, % viability, estimated by Trypan blue dye exclusion.

*RAM-Rh, spleen cells were incubated in rhodamine-labelled rabbit-anti-mouse Ig for 45 min at 37 °C and washed three times.

Other details as in Fig. 1.

period after anti-Ig treatment was increased to 72 h, to determine whether or not the impairment of SIg regeneration was durable. No receptor regeneration of CY-treated B cells was observed even after 72 h of culture, despite cell viabilities which were comparable to those of control groups (Table 2).

It has been suggested that CY-induced tolerance involves death of lymphocytes triggered by antigen either because of a block in their normal differentiation sequence or a selective vulnerability of the S or G₂ phases of the cell cycle⁸. This view is weakened by the finding that CY promotion of tolerance with T-independent antigens depends on their tolerogenicity rather than immunogenicity (ref. 5 and J. G. Howard and C. Hale, in preparation). An attractive and likely alternative is that the B cell tolerance-promoting effect of CY is due to an impairment of SIg receptor regeneration following interaction with multivalent antigens, assuming that the sequelae are similar to those following anti-Ig interaction. The fact that other immunosuppressive drugs seem incapable of reproducing this effect and of promoting tolerance strongly supports such a view. Moreover, the finding that B cells from CY-injected mice containing endocytosed rhodamine-labelled anti-Ig were still abundantly present after 24 h of culture further substantiates a lack of cytotoxic deletion.

Anti-Ig treatment of immature B cells in foetal liver, neonatal spleen and adult bone marrow cells^{10,11} causes an irreversible disappearance of SIg which is not found with mature B cells. This may explain the ease with which immature B cells are tolerised by multivalent antigens¹⁰. A similar result has been obtained by the prolonged exposure of B cells to certain polymeric antigens^{11,12}. We suggest that CY induces a temporary reversion to a state of functional immaturity analogous to that of B cells in foetal or newborn mice, during which their interaction with antigen leads to tolerance alone. We are currently seeking further evidence of indissociability between impaired SIg receptor regeneration and reversible immunosuppression of B cells induced by CY, as well as the nature of the underlying metabolic events.

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Received 17 October; accepted 30 November 1977.

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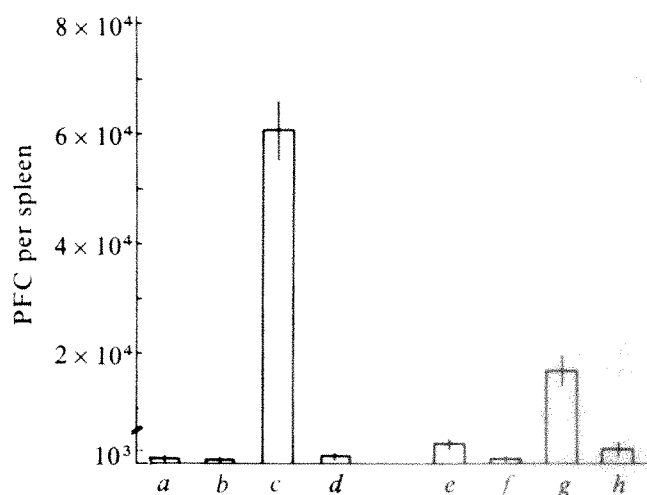
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Specific immune response enhancing factor in serum of immunised mice

ONE of the central problems of immunology is the mechanism by which regulatory thymus derived T cells—T helper, T amplifier and T suppressor cells—interact with each other and with the precursor cells of the antibody-forming B lymphocytes. Various mediators produced by T cells acting directly or indirectly on B cells have been described. Some of these factors have been shown to enhance^{1–4} or to suppress^{5,6} the function of B cells either in an antigen-specific^{1,2,5} or in a nonspecific^{3,4,6} manner. Immune response-enhancing factors produced *in vitro* have been shown to replace T helper or T amplifier cell function in thymus-dependent antibody formation *in vivo*^{1,2} and/or *in vitro*^{1–4}. We show here that the serum (S₄) of mice injected 4 h previously with a super-optimal dose of sheep red blood cells (SRBC) contains an antigen-specific principle—specific immune response enhancing factor (SIREF)—which enhances the humoral immune response up to 60-fold when injected before a sub-optimal antigen dose into mice but not in mice injected with an optimal dose of antigen (2 × 10⁸ SRBC). SIREF could not be produced in athymic nude mice and SIREF produced in BALB/c (H₂d) or C57Bl (H₂b) mice acted in either strain, but not in athymic mice (Table 1). These results indicate that SIREF(1) may be released from T cells (or from B cells which require T-cell cooperation), (2) acts through the H₂ barrier, and (3) does not act directly on B cells.

As shown in Fig. 1, SIREF is antigen specific—if produced

Fig. 1 Antigen specificity of SIREF. BALB/c mice were injected i.v. with 10⁹ SRBC or 10⁸ HRBC (horse red blood cells). Serum collected 4 h later (S₄ SRBC or S₄ HRBC respectively) was injected i.v. (0.1 ml per mouse) in mice immunised with either SRBC (10⁵ SRBC per mouse i.v.) or HRBC (5 × 10⁴ HRBC per mouse i.v.). S₄ was given 2 h before the antigens. Controls were injected with either S₄ SRBC, S₄ HRBC, 10⁵ SRBC or 5 × 10⁴ HRBC. The IgM PFC in the spleen of the mice were detected 6 d later. Each value represents the mean of PFC per spleen detected in 6 animals on day 6. The vertical bars represent the standard deviation of the mean. HRBC-immunised mice were tested for anti-HRBC-PFC, SRBC immunised mice were tested for anti-SRBC-PFC. a, SRBC (10⁵); b, S₄ SRBC; c, SRBC (10⁵) + S₄ SRBC; d, SRBC (10⁵) + S₄ HRBC; e, S₄ HRBC; f, SRBC (5 × 10⁴) + S₄ HRBC; g, HRBC (5 × 10⁴) + S₄ SRBC; h, HRBC (5 × 10⁴) + S₄ SRBC.



in SRBC-injected mice SIREF enhances the immune response in SRBC immunised recipients but not in mice immunised with horse red blood cells (HRBC) and vice versa. The enhancing effect of S_4 HRBC was, however, significantly less marked than that found in animals receiving S_4 SRBC and SRBC. Rabbit anti-SRBC antibody bound covalently to Sepharose removed up to 90% of SIREF activity from S_4 SRBC (T.D. and H.N., in preparation). This finding again shows the specificity of SIREF and suggests that SIREF has to contain such antigenic determinants.

SIREF is not simply an antigen or an immunogenic degradation product of SRBC. This view is supported by the following findings: (1) 0.4 ml of S_4 derived from either strain of mice was even less effective than 0.1 ml of S_4 in enhancing the plaque-forming cell (PFC) response, (2) when injected alone 0.4 ml S_4 was no more immunogenic (280 ± 93 PFC) than 0.1 ml of S_4 (314 ± 119) as tested 6 d later and (3), serum derived from antigen-injected nude mice did not show significant SIREF activity.

SIREF does not alter the initiation of the immune response but enhances and prolongs the antibody formation (Fig. 2). The effect of the factor in the primary immune response is restricted to the IgM response. Preliminary experiments (data not shown) indicate that SIREF counteracts a mechanism which determines both the magnitude and persistence of the IgM response. A similar activity has been described by Henry and Jerne⁷, who obtained identical results injecting an active principle isolated from the serum of SRBC-immunised mice 4 d after immunisation. They claimed that the serum activity was due to IgM anti-SRBC antibodies present in their preparation. In our experiments, serum containing SIREF obtained 4 h after immunisation was completely free of anti-SRBC antibodies (as tested by anti-SRBC antibody titration) and SIREF could be detected (in a much smaller amount) up to 4 d after immunisation. We suggest that the 'regulation of the immune response by IgM antibodies' as proposed by Henry and Jerne should in fact, to accommodate our findings, be considered as 'regulation of the IgM response by SIREF'. This suggestion is further supported by the findings (1) that SIREF is not dialysable, (2) that in ammonium sulphate treatment of serum the

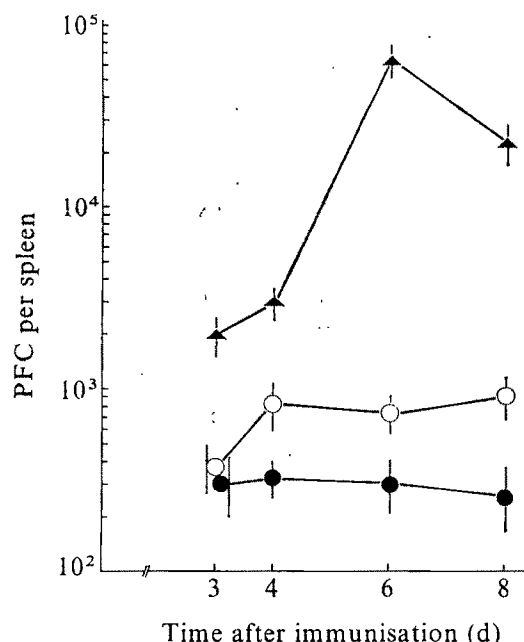


Fig. 2 Effect of SIREF on the kinetics of the IgM response. BALB/c mice 6–8 weeks old were injected with 10^8 SRBC; 0.1 ml serum collected 4 h later (S_4) was injected i.v., 2 h before immunisation of syngeneic animals with 10^6 SRBC i.v. Controls were injected with either S_4 or 10^8 SRBC i.v. At the times indicated direct IgM and indirect IgG PFC were assayed by the Jerne technique in the modification of Cunningham and Szenberg¹⁰ and as described in detail previously¹¹. Each point represents the mean of direct PFC detected in the spleens of 6 animals \pm standard deviation. IgG PFC could not be detected in either group of mice. Anti-SRBC hemolysine titers paralleled the PFC response presented here (Data not given here). ▲, S_4 +SRBC; ○, SRBC; ●, S_4 .

SIREF coprecipitates with the globulin fraction, and (3), SIREF was enriched in the conditions used by Henry and Jerne⁷ (H.N. and T.D., in preparation).

There are some similarities between SIREF and a serum factor described by Paraskevas *et al.*^{8,9}, who suggested that their factor is an antigen–IgM antibody complex released from B cells, cytophilic for a subset of T cells. This assumption was supported by the finding that spleen T cells derived from mice injected with SRBC 6 h previously and subsequently transferred into irradiated syngeneic animals enhanced the T cell-dependent IgG response (up to sevenfold) and the IgM response (up to two- to threefold) to SRBC. Paraskevas *et al.* assumed that at the time of the transfer the T cells were already armed with antigen–antibody complexes. SIREF, in contrast, does not affect the primary IgG response to either dose of SRBC but enhances dramatically (up to 60-fold) the IgM response when injected in normal animals immunised with a suboptimal antigen dose. Injection of SIREF does not induce immunological memory but injection of SIREF with a suboptimal dose of antigen induces development of memory cells of IgG type in a much higher amount than injection of the animal with the antigen alone (data not shown).

Several questions remain to be clarified: (1) is SIREF identical with Henry and Jerne's factor believed at that time to be IgM anti-SRBC antibody? (2) Is SIREF identical with the serum factor of Paraskevas *et al.*, and the discrepancies detailed above due only to differences in the ability to produce and/or to test the factor activity? (3) Are the three factors essentially identical, but according to our data released from T cells (T cell–receptor antigen complex) rather than from B cells (antigen–antibody complex)? The similarities in the production of SIREF, the factor obtained by Henry and Jerne or by Paraskevas and Lee and the finding that SIREF could not be produced in mice lacking functioning T cells (nude mice) seem to support the latter possibility. However, the contribution of

Table 1 T-cell dependence of production and action and the lack of H_2 -restriction of SIREF

S_4 donor	S_4 recipient	PFC response per spleen
BALB/c	BALB/c	$64,560 \pm 7,544$
BALB/c	C57Bl	$47,200 \pm 9,512$
BALB/c	nu/nu	257 ± 109
C57Bl	C57Bl	$36,480 \pm 7,651$
C57Bl	BALB/c	$73,640 \pm 11,459$
C57Bl	nu/nu	203 ± 77
nu/nu	nu/nu	263 ± 141
nu/nu	BALB/c	$1,329 \pm 273$
nu/nu	C57Bl	$1,174 \pm 309$
Recipient of 10^6 SRBC	S_4 donor and recipient	PFC response per spleen
BALB/c	—	841 ± 176
C57Bl	—	689 ± 251
nu/nu	—	224 ± 64
—	BALB/c (0.1 ml)*	314 ± 119
—	BALB/c (0.4 ml)	280 ± 93
—	C57Bl (0.1 ml)	347 ± 88
—	C57Bl (0.4 ml)	360 ± 65
—	nu/nu (0.1 ml)	293 ± 141
—	nu/nu (0.4 ml)	224 ± 64

BALB/c, C57Bl or athymic nude mice were injected with 10^6 SRBC i.v. Serum collected 4 h later (S_4) was tested for SIREF activity and for its immunogenicity on injection i.v. either S_4 2 h before immunisation of the mice with 10^6 SRBC i.v. or S_4 alone. S_4 derived from either donor strain was tested in either recipient strain. The values represent IgM PFC detected on day 6 in the spleen of 6 mice per group \pm s.d.

*Volume of S_4 injected shown in parentheses.

accessory cells in production and/or action of SIREF cannot be excluded.

We thank Mrs M. V. Odenwald for technical assistance.

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Received 19 July; accepted 15 November 1977.

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Transfer of the marker for morphologically transformed phenotype by isolated metaphase chromosomes in hamster cells

IN spite of extensive investigations of the process of carcinogenesis *in vitro*, the basic mechanisms of the primary events are poorly understood¹. In general, it is found that the first manifestation of the transformed phenotype involves morphological alteration of the cells. This is usually followed by an ill-defined process resulting in several further changes in phenotype including the ability of the cells

metaphase chromosome⁴⁻⁹. Most of the initial work was done with the genes for hypoxanthine phosphoribosyl transferase and thymidine kinase, but dominant markers such as resistance to methotrexate and ouabain can also be so transferred⁹, as can the wild-type alleles for three auxotrophic markers¹⁰. In seeking to delineate the genetic elements of carcinogenesis, we have examined the ability of purified metaphase chromosomes from Chinese hamster ovary (CHO) cells to transfer the first recognisable phenotype observed during cell carcinogenesis, that is, morphological transformation. Because of the low frequency of chromosome transfer, an appropriate method for selection of cells (transferees) which have incorporated the relevant markers from the background of recipient cells is necessary. Our experiments were based on the assumption that if transformation gene(s) could be transferred to senescent cells, then they should be able to produce colonies, whereas the majority of the cells which do not receive the appropriate marker, will not form colonies. The results presented here show that senescent hamster cells can be rescued and become transformed by transfer of purified metaphase chromosomes from CHO cells; the frequency of this event is similar to that of single gene markers.

Primary Chinese hamster lung cells were subcultured through five generations, and cloned. When the cloned cultures had reached confluence (~ 15 further generations), they were split into two subcultures, in 100-mm plates, and grown again. At the end of this time the cells were growing slowly, and were obviously in senescence. As might be expected, each clone gave rise to different numbers of cells at this stage. Nevertheless, in this way, a number of sets of two plates, each pair containing similar numbers of cells at the same level of senescence, were available as recipients. Purified metaphase chromosomes were isolated from CHO cells and aliquots added to one plate from each clone, as previously described^{2,9}. The plates were incubated at 37 °C in α -medium¹¹ containing 15% FCS for 2 d. Cells were then trypsinised, counted, replated in the same

Table 1 Rescue of Chinese hamster lung (CHL) cells from senescence after treatment with purified metaphase chromosomes of CHO cells

Donor for chromosomes*	No. of cells plated $\times 10^5$ CHL clone no.†						No. of colonies obtained CHL clone no.‡						No. of colonies per no. of cells or cell equivalent chromosomes $\times 10^6$
	1	2	3	4	5	6	1	2	3	4	5	6	
1 CHO	3	5	10	1	2	4	2	6	13	1	0	5	10.4
2	4	4	10	2	3	5	0	0	0	0	0	0	<0.4
3 CHO							0	0	0	0	0	0	<0.1

*A total of 2×10^6 cell equivalent chromosomes was added per plate.
†At day 2 after treatment cells treated with chromosomes (line 1) or untreated cells (line 2) were trypsinised and counted. The cells were plated in 20 ml of α -medium containing 15% FCS, at a concentration of up to 5×10^5 cells per 100-mm plate. Incubation of chromosomes alone (line 3) was continued in the above medium.
‡Colonies obtained after incubation at 37 °C for 3 weeks.

to grow in agar (aga⁺), and to produce tumours in animals. In part because of the variety of phenotypes observed, but also because of the lack of suitable methods of analysis, it has been difficult to examine the process of transformation at the genetic level. We have recently initiated experiments designed to use chromosome transfer techniques to attempt such a genetic analysis^{2,3} and work in several laboratories has shown that single gene markers can be transferred from cell to cell at frequencies of 10^{-6} - 10^{-7} by means of the

medium and incubated at 37 °C for 3 weeks. Colonies were then counted and isolated using a stainless steel cylinder and trypsinisation. The results of the first such experiment are presented in Table 1 which shows that colonies were obtained in five of the six cultures treated with metaphase chromosomes (line 1), whereas no colonies were obtained when the senescent cells were plated without previous addition of metaphase chromosomes (line 2). No colonies were obtained when metaphase chromosomes were

Table 2 Rescue of Chinese hamster lung (CHL) cells from senescence after treatment with purified metaphase chromosomes of CHO cells

Donor for chromosomes*	No. of cells plated $\times 10^5$ CHL clone no.†					No. of colonies obtained CHL clone no.‡					No. of colonies per no. of cells or cell equivalent chromosomes $\times 10^6$
	11	12	13	14	15	11	12	13	14	15	
1 CHO	6	4	10	10	1	3	2	10	7	0	7.1
2 CHLP6	6	8	8	9	2	0	0	0	0	0	<0.4
3 CHOP6	7	5	8	8	2	0	0	0	0	0	<0.4
4	5	4	9	10	2	0	0	0	0	0	<0.4
5 CHO						0	0	0	0	0	<0.1

* , †, And ‡ as in legend to Table 1.

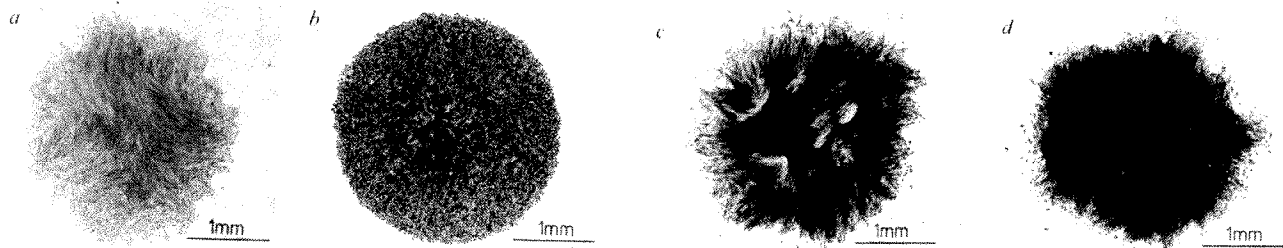


Fig. 1 Normal and transformed colonies. Normal recipient Chinese hamster lung cells (a), donor CHO cells (b) and two phenotypically different morphologically transformed Chinese hamster lung cells with metaphase chromosomes of CHO cells (c, d).

added to the plates in the absence of recipient cells (line 3). The results of a similar experiment, with two additional controls, are shown in Table 2. Again, colonies were obtained when CHO chromosomes were added to the senescent cells (line 1) and no colonies were found in the absence of chromosomes (line 4) and in the absence of recipient cells (line 5). We also prepared metaphase chromosomes from two primary strains developed from Chinese hamster lung and Chinese hamster ovary, both in their sixth transfer passage (1:4 splits), CHLP6 and CHOP6, respectively. As may be seen in Table 2 (lines 2 and 3), these chromosomes were also unable to rescue senescent cells.

The experiments described in Tables 1 and 2 provide strong evidence that CHO metaphase chromosomes can rescue senescent cells. Figure 1 shows the morphology of clones from two of these transferent clones, as well as that of the donor and primary recipient cells. It is clear that the rescued cells have been altered morphologically and have the appearance of transformed cells. This transformed phenotype is maintained on continued culture of these clones. Four of the transferents were karyotyped in detail with trypsin-Giemsa banding. Of 26 cells examined, 60% had a normal diploid karyotype, 15% showed loss of one or more chromosomes, and 25% showed structural rearrangements.

spontaneous transformation in the recipients, and also tend to rule out any mechanism of 'induction' of transformation in recipients due to addition of chromosomal material.

The frequency of appearance of transferents in all of the experiments (Tables 1-3) is similar to that observed for a variety of single gene markers^{2,9,10}. This fact, in addition to the localisation of the activity to one size class argues for the view that the transformation of recipient cells by metaphase chromosomes is caused by the transfer of a single gene.

Although the transferent cells obtained in these experiments show a transformed morphology, we have found that they do not form colonies in agar (aga⁻) or tumours when injected into recipient animals², but they can be converted into cells which do form colonies in agar (aga⁺) and which can produce tumours in animals, by a second addition of CHO metaphase chromosomes, and appropriate selection². It has not been possible to produce aga⁺ cells directly by addition of CHO chromosomes to primary cells. In terms of their ability to be converted to aga⁺, the primary transferents behave identically to cells which have been transformed by benzo(a)pyrene, or cells which have transformed spontaneously in culture². These results provide further confirmation that the rescued senescent cells are transformed, and that a gene involved in a first step in

Table 3 Rescue of Chinese hamster lung (CHL) cells from senescence after treatment with fractionated metaphase chromosomes of CHO cells									
Chromosomal fraction*	No. of cells plated × 10 ⁵ CHL clone no.†				No. of colonies obtained CHL clone no.‡				No. of colonies per no. of cells or cell equivalent chromosomes × 10 ⁶
	21	22	23	24	21	22	23	24	
1 Large(A)	7	5	10	6	8	7	12	8	12.5
2 Medium(B)	4	6	9	7	0	0	2	0	0.8
3 Small(C)	5	4	8	7	1	0	0	0	0.4
4	5	4	9	8	0	0	0	0	<0.4
5 A+B+C					0	0	0	0	<0.2

*. † and ‡ as in legend of Table 1.

From these results, we conclude that the addition of CHO metaphase chromosomes to senescent cells provides genetic information resulting in the transformation phenotype, and in rescue of senescence. Further support for this view has come from an experiment with fractionated chromosomes. Previously, we showed that metaphase chromosomes could be separated into three size classes in sucrose gradients (large A, medium B, small C), and that individual markers could be localised to specific size classes^{9,10}. Metaphase chromosomes from CHO cells were fractionated in this way, and each fraction was tested for the ability to rescue senescent recipient hamster cells. As may be seen in Table 3, lines 1-3, activity was found in the large size (A) class of chromosomes but few or no colonies were obtained with the other fractions. As before, no colonies were obtained in the absence of chromosomes (line 4) or recipient cells (line 5). Transferent colonies obtained in this experiment were also transformed in phenotype.

Thus, the ability to transform cells behaves similarly to other genetic markers and can be located on a specific size class of chromosomes.

The negative findings with the B and C chromosome size classes provides further evidence that the transformed colonies obtained result from addition of donor chromosomal material, rather than

transformation has been identified. The successful isolation of such transferents also provides evidence that this gene acts dominantly. Our results do not, however, show that this is the only gene involved in the primary transformation step. Further experiments involving a variety of donors, and recipients, will be required to examine questions of this kind.

Dr R. Worton provided useful comments on the manuscript, in addition to help with the analysis of the karyotypes; Miss Nancy Stokoe provided technical assistance. The research was supported by the MRC of Canada, the NCI of Canada, and the US NIH. D.A.S. is a recipient of an MRC (Ottawa) postdoctoral fellowship.

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Received 26 September; accepted 14 November 1977.

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Deficient recovery from potentially lethal radiation damage in ataxia telangiectasia and xeroderma pigmentosum

THE enhanced survival that occurs when mammalian cells are maintained in a density-inhibited state for a short time after treatment with X rays, ultraviolet light or drugs has been termed potentially lethal damage repair (PLDR)¹⁻³. It is analogous to liquid-holding recovery in bacteria and yeast, and has been studied using a variety of agents in different cell lines³⁻⁵ and in malignant tumours^{6,7}. To investigate the relationship between this cellular recovery phenomenon and repair at the molecular level, we have examined PLDR in human diploid cell strains with known molecular repair defects. We report here that xeroderma pigmentosum (XP) skin fibroblasts show no PLDR following ultraviolet light irradiation, whereas ataxia telangiectasia (AT) skin fibroblasts are specifically deficient in PLDR following X-ray irradiation. The results suggest that, as in bacterial cells, this cellular recovery phenomenon does reflect molecular DNA repair—probably the excision repair pathway.

XP is an autosomal recessive disease characterised by extreme sensitivity to ultraviolet light and leading to the development of multiple malignant skin tumours. Skin fibroblasts from these patients show a deficiency in the excision repair of ultraviolet light-induced DNA base damage⁸. Five complementation groups designated A to E, as well as a variant group of XP patients, have been identified. AT is an autosomal recessive disease characterised by cerebellar ataxia, telangiectasia of the bulbar conjunctiva and a predisposition to malignant tumours, especially of the reticulo-endothelial system. When treated for malignancy by irradiation, AT patients have in some cases demonstrated a clinical radiosensitivity. Also, skin fibroblasts derived from these patients are unusually sensitive to X rays *in vitro*⁹ and a defect in the repair of gamma irradiation induced DNA damage has been described in some strains¹⁰.

We have examined the capacity of skin fibroblast cell strains derived from patients with XP and AT to perform

PLDR following either ultraviolet light or X-irradiation. The results were compared with those for a normal diploid fibroblast cell strain. PLDR refers to the enhancement in cell survival which occurs in density-inhibited plateau phase cultures when assay for the surviving fraction by subculture at low density is delayed for several hours after irradiation¹.

The D_0 (inverse of the slope) values of the survival curves following irradiation of these cell strains in exponential growth are shown in Table 1. As previously reported⁹, AT was markedly more sensitive to gamma irradiation than either the normal strain (Li 106) or the XP (A) strain. XP strains A and C, however, were significantly more sensitive to ultraviolet light than either AT or Li 106. Figure 1 shows the results of X-ray PLDR experiments in which Li 106, AT and XP fibroblasts were irradiated in the density-inhibited plateau phase of growth; following X-ray irradiation, cells were subcultured at regular time intervals over 0-24 h to assay for colony forming ability. Doses were chosen to yield approximately the same surviving fraction. Recovery in Li 106 and XP cells is manifested by a maximum increase in survival of 4- and 5-fold, respectively (Fig. 1). In the case of X-ray repair deficient AT cells, however, maximum recovery was by a factor of 1.5, at a survival level considerably lower than for XP or Li 106 (Fig. 1). As the enhanced survival in PLDR is associated with a change in the slope of the survival curve, one would expect a greater increase in PLDR at lower survival fractions¹.

Figure 2 shows the results of ultraviolet light PLDR experiments in which the normal, XP (group A), and AT cells were irradiated in the density inhibited state, then subcultured 0-32 h later. Recovery in the AT and normal cell strains was approximately 3-fold and 5-fold, respectively, in this experiment (Fig. 2); ultraviolet light-induced PLDR took place more slowly than X-ray PLDR (Fig. 1) as is also the case for molecular (excision) repair following the two types of radiation. There was no recovery in the XP strain; the slight decline in survival seen with short recovery intervals was a consistent finding. Ultimate recovery ratios representing the results of at least three separate experiments for each cell strain following X-irradiation and ultraviolet light are shown in Table 1. No recovery was observed in XP cells from complementation groups A and C following exposure to ultraviolet light, and recovery was significantly reduced in X-irradiated AT cells. Thus, cells which are deficient in the excision repair of ultraviolet light damage did not perform PLDR following ultraviolet light exposure, and X-ray repair deficient cells (AT) seem to have a markedly reduced capacity for PLDR following X-ray exposure. The results with Li 106 are

Table 1 Radiation sensitivity and repair of potentially lethal damage in human diploid fibroblast strains

Cell strain and source	Clinical classification	Cloning efficiency	D_0 (X ray)	Enhancement after X ray	Survival D_0 (ultraviolet light)	Survival enhancement after ultraviolet light
Li 106 (Primary culture)	Normal skin fibroblasts	1.0-6.3%	149±7	4.13±0.03	31±6	4.33±0.34
CRL 1343 (ATCC)	Skin fibroblasts from ataxia telangiectasia	0.7±3.5%	46±3	1.78±0.13	29±3	3.10±0.55
XP12BE (ATCC CRL 1223)	Skin fibroblasts from xeroderma pigmentosum (XP) (group A)	7.9-23.0%	160±17	4.63±1.04	6±1	0.96±0.15
XP8BE (IMR-GM 671)	XP skin fibroblasts (group C)	8.3-16.9%	—	—	8±1	0.91±0.21
XP4BE (ATCC CRL 1162)	XP skin fibroblasts (variant)	0.7-1.2%	—	—	20±2	2.30±0.40

D_0 is inverse of slope of complete survival curve; exponentially growing cells were trypsinised, seeded at appropriate numbers into triplicate 10-cm plastic dishes, and irradiated 18 h later. The D_0 values were derived from least squares linear regression analysis of at least three experiments. PLDR experiments were carried out as described in Figs 1 and 2. Numbers represent mean survival enhancement ±1 s.e.m. in at least three separate experiments for each cell strain (two experiments for XP (c) and XP variant strains determined after 6 h for X-irradiation and after 24 h for ultraviolet light irradiation).

characteristic of those with five normal human diploid fibroblast strains tested concurrently in our laboratory.

XP variant cell strains show near normal sensitivity to cell killing by ultraviolet light and a normal excision repair capacity, but an apparent deficiency in a post-replication repair process¹¹. The results of ultraviolet light PLDR experiments carried out with an XP variant strain are also shown in Table 1. While groups A and C XP cells were deficient in excision repair, the variant cells were able to carry out PLDR following ultraviolet light irradiation.

We have demonstrated that cells deficient in molecular repair following X-ray (AT) or ultraviolet light (XP) exposure are similarly defective in PLDR. This report is the first to correlate specific DNA repair defects with impairment of a cellular recovery process in mammalian cells. The results suggest that PLDR as a general phenomenon in density-inhibited cells reflects molecular repair processes, which in turn are reflected by an increase in survival. Furthermore, the fact that XP variant cells can perform PLDR while other XP cells cannot suggests that ultraviolet light induced PLDR may specifically reflect the excision repair pathway.

Both AT and XP patients have a predisposition to develop malignancies, and deficiencies in these particular repair systems may be relevant to this susceptibility. Actively proliferating cells in renewal systems may turn over and be lost from the body before they express neoplastic development. However, spontaneous or induced DNA damage in resting stem cells might be repaired mainly by mechanisms such as those reflected by PLDR. These mechanisms appear to act particularly efficiently in non-proliferating cells. Since the stem cell is the likely target as the origin of neoplastic development, deficiencies

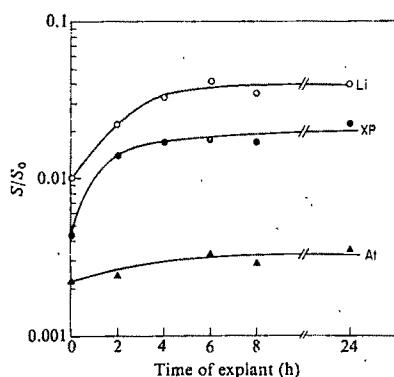


Fig. 1 Repair of X-ray-induced potentially lethal damage ○, Li 106 normal skin fibroblasts ●, CRL 1223, skin fibroblasts from a patient with xeroderma pigmentosum (complementation group A); ▲, CRL 1343, skin fibroblasts from a patient with ataxia telangiectasia. Cultures were maintained at 37 °C in Eagle's minimal essential medium (Gibco F-15) supplemented with 15% foetal calf serum (Microbiological Associates), D-glucose (900 mg l⁻¹), sodium pyruvate (0.66 mg l⁻¹) and chlortetracycline (Aureomycin, Lederle, 50 µg ml⁻¹) in a 5% CO₂ atmosphere. Cells from each strain were studied at similar passage levels in cultures (ranging from the 4th to 28th mean population doubling). Cells were grown to confluency in 6-cm dishes. The culture medium was renewed daily for 3 d after initial confluency and the experiment was performed on the 4th day. Plates were irradiated at room temperature with a 220 kVp GE Maximar unit operated at 15 mA with a dose rate of 80 rad min⁻¹. XP and normal fibroblast cultures were given 700 rad, AT cultures 350 rad. Following irradiation, plates were returned to the incubator. Single plates were removed from the incubator at regular time intervals, trypsinised (0.25% trypsin in Ca²⁺ and Mg²⁺-free Earle's balanced salt solution), and cells seeded in 10-cm dishes in triplicate. The number of cells seeded per dish ranged from 20,000 to 40,000, depending on cell strain and experimental conditions. Medium was changed after 7 d, and after approximately 14 d, cells were fixed, stained with 0.5% methylene blue and scored. Colonies containing 50 or more cells were scored as survivors. The figure depicts a single representative experiment. S, Number of cells surviving at a given time; S₀, number of cells at zero time.

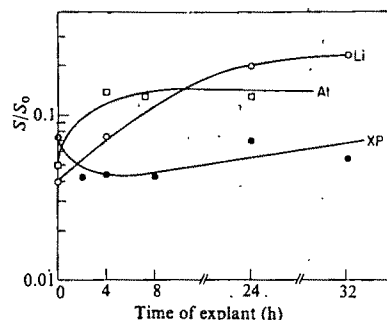


Fig. 2 Repair of ultraviolet light induced potentially lethal damage ○, normal skin fibroblasts; □, AT fibroblasts; ● XP fibroblasts. Cultures were maintained and grown to confluency as described in Fig. 1. On the 4th day, cultures were rinsed with Earle's Balanced salt solution, the solution removed, and the cultures irradiated with a 254 nm light source at a dose-rate of 4.5 erg mm⁻² s. Normal and AT cultures were exposed to 120 erg mm⁻², XP cells 20 erg mm⁻² except XP variant (60 erg mm⁻²). Following irradiation, conditioned medium was added, and the dishes returned to the incubator. Cultures were trypsinised at regular intervals and cells seeded into 10-cm dishes in triplicate. The dishes were maintained, fixed, stained and scored as described in Fig. 1.

in a repair process which is usually thought to be error free (excision-repair) might lead to the accumulation of mutational damage in the DNA and lead to the ultimate expression of malignant change.

We suggest that AT cells are deficient in X-ray PLDR while XP cells are deficient in ultraviolet PLDR, and that this cellular recovery phenomenon reflects molecular repair processes. Deficiencies in this type of repair may contribute to the general predisposition to the development of malignancies in these patients.

This work was supported by a grant from the US NCI.

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Butyric acid suppression of the *in vitro* neoplastic state of Syrian hamster cells

BUTYRIC acid has been shown to induce morphological and biochemical differentiation in a variety of cells in culture¹⁻⁸. Although the mechanism of action of this short chain fatty acid remains to be understood, changes induced by butyric acid have included: (1) growth inhibition and morphological alterations in HeLa cells, Chinese hamster ovary cells, and neuroblastoma cells²⁻⁴; (2) increases in tyrosine hydroxylase activity and adenylate cyclase activity in neuroblastoma cells^{1,3}; (3) induction of erythroid differentiation in erythroleukaemic cells⁶; and (4) induction of functional β -adrenergic receptors in HeLa cells^{7,8}. We reasoned that the

induction of these differentiated functions might be associated with a concomitant suppression of neoplastic properties. Therefore, we have investigated the effects of butyric acid on the aberrant morphology, anchorage-independent growth, and enhanced fibrinolytic activity of a highly tumorigenic Syrian hamster fibroblast cell line, BP6T (refs 9, 10). We have found that butyric acid causes reversible, specific suppression of each of these altered *in vitro* properties frequently associated with neoplasia. This system could be of value for the investigation of the mechanism of the cellular control of the neoplastic state.

We studied the effects of butyric acid on BP6T cells and two clonal isolates, BP6T-But 1 and BP6T-But 2, derived from BP6T cells. These two clonal strains were obtained by single colony isolation following two subculturings in medium supplemented with 2 mM butyric acid (final pH 7.2), and maintained in this medium unless otherwise stated. The addition of butyric acid to the culture medium produced a slight decrease in the growth rate and cloning efficiency of BP6T cells. Table 1 summarises the growth properties of BP6T cells and the two clonal isolates in unsupplemented medium and in medium containing butyric acid. Butyric acid slowed the doubling time by 4–7 h, and reduced the cell saturation density 20–70%, although the degree of response differed among the three cell strains. These suppressive effects on growth were reversed on removal of the butyric acid (Table 1). Since the tumorigenic BP6T cells exhibit a high plating efficiency on solid surfaces in liquid medium at low cell density, we investigated the effects of butyric acid on this property. BP6T, BP6T-But 1, and BP6T-But 2 cells were reduced in their abilities to form colonies when inoculated at 100 cells per plate in liquid medium containing butyric acid (Table 1). The cloning efficiencies ranged from 14% to 25% in medium containing butyric acid, contrasting with cloning efficiencies of 65–85% in normal medium. Although a diminution of the cloning efficiency resulted from the addition of butyric acid, the efficiency of cloning achieved for all three strains

remained much greater than that obtained with untransformed Syrian hamster embryo cells, which require plating densities of greater than 10^3 cells per 100-mm dish to produce any colonies.

In contrast to the mild effect on the general growth properties mentioned above, butyric acid added to culture medium produced profound effects on the colonial morphology, anchorage-independent growth, and fibrinolytic activity of BP6T cells. The random, criss-crossed orientation of spindle-shaped cells within a BP6T colony grown in the absence of butyric acid (Fig. 1a) is characteristic of Syrian hamster fibroblast lines transformed *in vitro* by chemical carcinogens^{11–14}. Dramatic alterations in this morphology were elicited in BP6T cells by a supplement of 2 mM butyric acid. In medium containing butyric acid, all colonies displayed morphologies similar to BP6T-But 1 and BP6T-But 2 (Fig. 1b and 1c), in that individual cells had a lower nuclear-cytoplasmic ratio and were orientated in a uniform, parallel pattern within the colony. This colony morphology is similar to that of untransformed Syrian hamster cells^{11–14}. To test the reversibility of this change, we plated cells of BP6T-But 1 and BP6T-But 2 into medium lacking butyric acid. In both cases, the transformed morphology of the colonies was restored.

The effect of butyric acid on anchorage-independent growth was examined by cloning in semi-solid agar^{9, 12, 15}. Following growth for 10 d in medium lacking butyric acid, BP6T cells form colonies at 50% efficiency following suspension of 200 cells⁹. When suspended in agar supplemented by 2 mM butyric acid at concentrations of 10^5 cells per plate, BP6T, BP6T-But 1, and BP6T-But 2 cells were unable to form colonies (Table 1). In contrast to its mild effect on cloning efficiency in liquid medium, butyric acid significantly inhibited anchorage-independent colony formation. To test the stability of the suppressive effect of butyric acid on anchorage-independent growth, the two clonal isolates were grown through 14 passages (approximately 54 population doublings) in liquid medium containing butyric

Table 1 Reversible effect of butyric acid on growth properties of Syrian hamster fibroblasts

Cell type*	Butyric acid concentration (mM)	Population doubling time† (h)	Cell saturation density‡ (cells cm ⁻² × 10 ⁵)	Cloning efficiency‡ (%)	Anchorage-independent cloning efficiency§ (%)
BP6T	0	13	5.5	85	50 (2 × 10 ⁵)
BP6T	2	20	2.0	15	< 0.001 (10 ⁵)
BP6T-But 1	2	20	1.7	14	< 0.001 (10 ⁵)
BP6T-But 1	0	15	4.0	65	32 (2 × 10 ⁵)
BP6T-But 2	2	16	4.0	25	< 0.001 (10 ⁵)
BP6T-But 2	0	12	5.5	80	40 (2 × 10 ⁵)
Syrian hamster embryo cells	0	14	2.0	2–6	< 0.001 (10 ⁵)

*BP6T, a tumorigenic Syrian hamster fibroblast line, was derived from a fibrosarcoma which formed in a newborn Syrian hamster following injection of cells of a benzo(a)pyrene-transformed Syrian hamster fibroblast line. Experiments were conducted with BP6T cells 4–15 *in vitro* passages following tumour isolation. BP6T sublines BP6T-But 1 and BP6T-But 2 were isolated from BP6T cells grown in the presence of an exogenous supplement of butyric acid (2 mM, pH 7.2) for two passages. Colonies which formed following plating of 50 BP6T cells into 100-mm plates containing butyric acid-supplemented medium were isolated and grown continuously in that medium. Syrian hamster embryo cells were derived from 11-d-old embryos; their growth properties and fibrinolytic activities were measured between passages 4 and 8 inclusive. Cell culture procedures used have been described previously^{9, 10}.

†The population doubling time was measured during exponential growth at cell densities 1/10 of the cell saturation density for each line. Cell saturation densities were determined in 25 cm² tissue culture flasks (Falcon). For the measurement of population doubling time and cell saturation density in the absence of butyric acid, cells from BP6T-But 1 and BP6T-But 2 were measured one passage following removal of the compound.

‡The cloning efficiency was determined by plating 100 cells into 100-mm × 20-mm (Falcon) optilux polystyrene dishes in 10 ml of medium with or without butyric acid (2 mM). Colonies which formed after 10–14 d were fixed, stained with Giemsa, and counted. The cloning efficiencies are expressed as percentages of the number of cells plated. The cloning efficiency of Syrian hamster embryo cells was determined using 0.5×10^4 – 2×10^4 cells as an initial inoculum.

§Anchorage-independent colony formation was measured by suspending cells in semi-solid agar medium containing 0.4% Difco bacto agar, 0.1% bacto-peptone, and 10% foetal calf serum dissolved in culture medium⁹. Suspended cells were incubated for 14 d to allow colony growth. The colony-forming efficiencies are expressed as percentages of the total number of cells suspended; the figures in parentheses are the number of cells suspended in each dish.

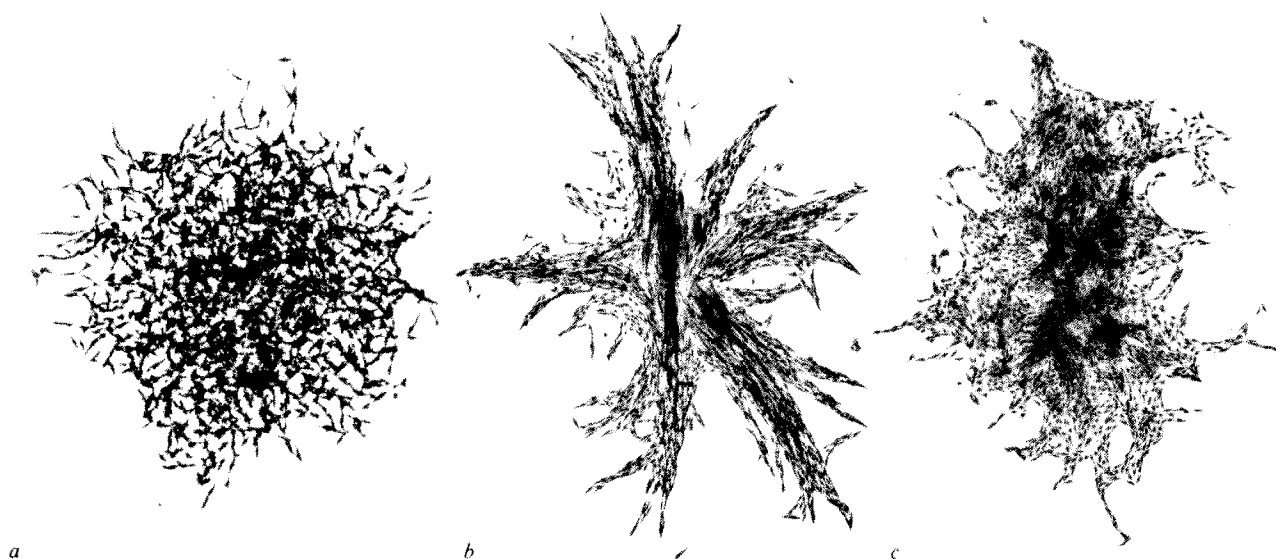


Fig. 1 Effect of butyric acid on colonial morphologies. *a*, BP6T colony formed in the absence of butyric acid, fixed and stained with 10% aqueous Giemsa; *b*, BP6T-But 1 colony (P12) formed in the presence of 2 mM butyric acid; *c*, BP6T-But 2 colony (P 12) formed in the presence of 2 mM butyric acid. All magnifications are $\times 40$.

acid. Following suspension of 10^5 BP6T-But 1 cells in agar containing 2 mM butyric acid, no growth was observed. In contrast, BP6T-But 2 cells partially regained the capacity for anchorage-independent growth, forming 150 colonies following suspension of 2.5×10^4 cells in agar containing 2 mM butyric acid. These results indicate both the stable nature of the suppression of anchorage-independent growth in the presence of butyric acid, and the possibility of obtaining strains which are resistant to this suppressive effect. The suppression of anchorage-independent growth was, however, reversed following removal of the butyric acid supplement. Omission of butyric acid from the culture medium and cultivation of BP6T-But 1 and BP6T-But 2 cells through two passages (approximately eight population doublings) resulted in near restoration of the anchorage-independent cloning efficiency to that of BP6T cells (Table 1).

Previous investigations have indicated that both the variant morphology of transformed lines, and anchorage-independent growth *in vitro* depend on cell-mediated fibrinolytic activity¹⁶⁻¹⁸. Quantitative assays of fibrinolysis have been used to measure the cellular production of the proteolytic factor, plasminogen activator, which cleaves plasminogen to its enzymatically active fibrinolytic form, plasmin^{10,19}. Elevated plasmin-mediated fibrinolysis is a property of BP6T cells and other tumorigenic Syrian hamster cell lines, but not of early passage Syrian hamster embryo cells^{10,16,17}. We examined the effect of butyric acid on both extracellular fibrinolytic activity, present in medium incubated with cells, and on intracellular fibrinolytic activity, present in cell lysates (Table 2 and Fig. 2). Fibrinolytic activity was measured by the extent of degradation of insoluble ^3H -fibrin to soluble ^3H -fibrinopeptides in a cell-free assay¹⁰. BP6T cells exhibited high levels of extracellular fibrinolytic activity, whereas BP6T-But 1 and BP6T-But 2 cells grown in the presence of 2 mM butyric acid produced low levels of extracellular activity, reduced by 85–95% compared with BP6T cells (in the absence of butyric acid) following growth of BP6T-But 1 cells for three passages (approximately 12 population doublings) in medium lacking butyric acid, however, the extracellular activity increased to 40% of the activity of the BP6T cells.

BP6T cells grown for 48 h in medium supplemented with 2 mM butyric acid exhibited an 80–90% reduction in intracellular fibrinolytic activity (Fig. 2). BP6T-But 1 cells grown

in the presence of butyric acid for 14 passages also exhibited reduced intracellular fibrinolytic activity (70–80% reduced compared with BP6T intracellular activity). Following cultivation of BP6T-But 1 cells through three passages in medium lacking butyric acid, the level of intracellular fibrinolytic activity in this strain rose to a level comparable with that of the parent BP6T cells. During this period, the capacity for anchorage-independent growth was restored (Table 1).

Aberrant morphology, anchorage-independent growth, and enhanced proteolytic activity are *in vitro* cellular properties which have been correlated with tumorigenicity.

Fig. 2 Reversible effect of butyric acid on intracellular fibrinolytic activity. The fibrinolytic activity in cell lysates was measured by the method of ^3H -fibrin degradation as described previously¹⁰. (One unit of fibrinolytic activity is as defined in Table 2). Results represent the means of duplicate determinations, following subtraction of radioactivity released by buffer alone (background). ●, BP6T cells; ○, BP6T cells cultured 48 h in the presence of 2 mM butyric acid; □, BP6T-But 1 cells cultured in the presence of 2 mM butyric acid; ■, BP6T-But 1 cells, butyric acid removed for 12 population doublings; ▲, Syrian hamster embryo cells, passage 3.

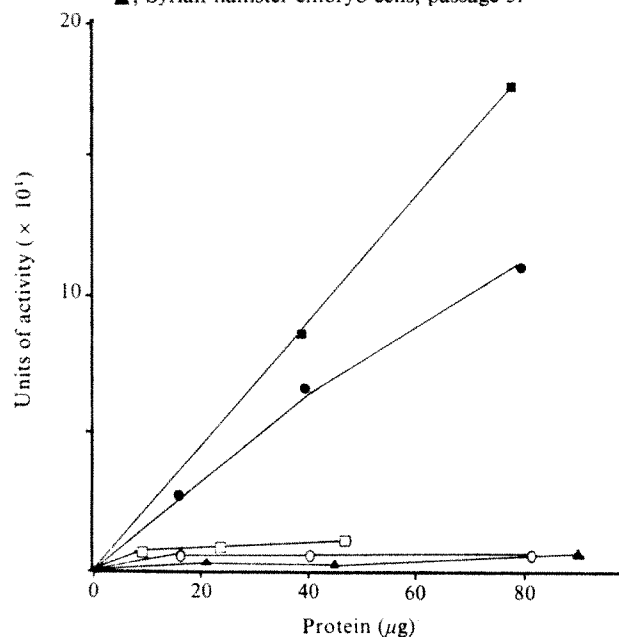


Table 2 Reversible effect of butyric acid on the fibrinolytic activities of Syrian hamster fibroblasts

Cell type	Butyric acid concentration (mM)	Extracellular fibrinolytic activity* (units per 2 ml culture medium)
Control (no cells)	0	3.1
Syrian hamster embryo cells	0	3.2
BP6T	0	4.2
BP6T-But 2	2	4.2
BP6T-But 1	2	6.1
BP6T-But 1	0	16

*The procedure for measuring extracellular fibrinolytic activity by the technique of ^3H -fibrin degradation has been described¹⁰. One unit of fibrinolytic activity is defined as that which solubilises 10% of the radioactivity release by 100 units of urokinase in 2 h at 37 °C. Values for extracellular activity represent the total units of fibrinolytic factor present in 2 ml of medium cultured in the presence of cells. The background activity observed with control culture medium (which was not incubated with cells) was 3.1 urokinase unit equivalents. The averages of duplicate determinations are reported. Addition of 2 mM butyric acid to medium from BP6T cells cultured in the absence of butyric acid had no significant effect on the extracellular enzyme activity.

The molecular processes, however, which control the expression of these phenotypes are not well understood. One approach toward defining these processes is to regulate the expression of specific phenotypes through the application of exogenous agents. Our results demonstrate that the expression of aberrant colony morphology enhanced fibrinolytic activity, and anchorage-independent growth can be regulated in transformed Syrian hamster cells by the addition of butyric acid to culture medium. Control experiments have shown that butyric acid has no effect on the protein concentration of cell lysates or the activities of two unrelated enzymes, hypoxanthine phosphoribosyltransferase and adenine phosphoribosyltransferase, which are extracted by the same procedure^{20,21} (data not shown). This suggests that the effects we have observed may reflect some degree of specificity of the action of butyric acid. As these phenotypic alterations can be observed during relatively short periods, kinetic analyses of the appearance and disappearance of specific cellular properties following the addition or removal of butyric acid should enable a determination of their interrelationships. An extension of these preliminary studies to an examination of changes in messenger RNA, proteins, and other macromolecules synthesised during the phenotypic reversals, and a determination of the mechanism of action of butyric acid may lead to a better understanding of cellular mechanisms regulating the neoplastic state.

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Actin filaments form the backbone of nemaline myopathy rods

NEMALINE myopathy, a congenital neuromuscular disease, is one of several muscle disorders in which apparently abnormal Z lines, or Z line-type structures emanating from Z lines, has been described¹⁻⁶. There has been considerable speculation concerning the chemical composition and structural arrangement of proteins in nemaline rods^{2,4,7-12}, but these features have remained unclear: part of this uncertainty is related to lack of understanding of the intact Z line. The only Z line constituent for which there is substantial evidence is α -actinin¹³⁻¹⁶. We have described the preparation and properties of a Ca^{2+} -activated neutral protease (termed CAF) from muscle that is highly specific in its activity towards myofibrillar proteins and structure^{17,18}. Addition of CAF to isolated myofibrils or to teased muscle fibrils releases undegraded α -actinin from the Z line, with no noticeable effect on myosin, actin, or the remaining myofibrillar structure. We report here the use of CAF as a dissection tool to strip away the dense, amorphous component of the nemaline rods, exposing an underlying set of longitudinal filaments running parallel to the long axis of the original rod. Decoration of these filaments with heavy meromyosin¹⁹ shows that the longitudinal filaments of nemaline rods are composed of actin.

Skeletal muscle containing nemaline rods was obtained by biopsy from a patient with congenital rod disease (second biopsy, case No. 1; see ref. 20 for clinical details) and stored at -60 °C until glycerination. It has been shown previously that glyceration does not alter fine structure of the rods^{7,12}. Typical morphology of the muscle consists of areas which contain rods with associated thin filaments interspersed with areas of myofibrils containing moderately widened Z lines (Fig. 1a). Rods are often found grouped together and frequently seem to be branched. There are thin filaments continuous with the longitudinal lines of the rod, as has been described previously^{7,12}. Small numbers of thick filaments usually surround the rod bodies, but are not continuous with it. The longest rod shown in Fig. 1a was 4.3 μm long; many locations in all sections sampled contained rods from ~2 to 6 μm long, and all rods demonstrated the five characteristic features described by Engel²¹. In particular, the rods display a set of periodic (140 Å) longitudinal lines running parallel to their long axis; they also show periodic (345 Å) lines perpendicular to their long axis.

A teased, glycerinated nemaline fibre treated extensively with CAF is shown in Fig. 1b. Digestion with CAF removes dense material of the Z line as reported previously; it also removes seemingly dense material from the rods and exposes longitudinal filaments with a uniform diameter (60-70 Å) which span the lengths of the original rods. Thus, their length is dependent on the length of the original rod. Removal of the dense, amorphous material coincides with disappearance of the perpendicular periodicity in the rods. The exposed longitudinal filaments are not as straight as the parallel longitudinal lines present in the original rods, presumably because of loss of supporting material along

their perpendicular axis. As a result, they often can be seen to associate with each other. Stromer *et al.*¹² observed a similar loss of straightness in longitudinal filaments of nemaline rods surviving extended (40 d), low ionic strength extraction.

Because the long, exposed filaments resemble thin filaments in diameter, because thin filaments run into or at least up to the edge of rods in their intact state^{7,12,21} (Fig. 1a), and because of the similarity in resistance to CAF shared by thin filaments¹⁸ and these filaments (Fig.



Fig. 1 *a*, Electron micrograph of a longitudinal section of nemaline muscle. Thin strips of muscle were glycerinated as described¹⁹. After thin sectioning, fibres were prefixed with 3% glutaraldehyde, postfixed in 1% osmium tetroxide, and embedded in Epon-Araldite. Sections were stained with uranyl acetate, followed by lead citrate, and examined in an RCA-EMU-4 microscope operated at 100 kV. Note the large rod body with its long axis parallel to the adjacent myofibril. Longitudinal lines parallel to the long axis of the rod are very evident. M, M line; Z, Z line. *b*, Electron micrograph of a longitudinal section of nemaline muscle after CAF treatment. Teased fibres from glycerinated muscle were incubated with CAF (0.16 mg ml⁻¹) in the presence of 66 mM KCl, 33 mM Tris-acetate, pH 7.5, 3.3 mM CaCl₂, 6.6 mM 2-mercaptoethanol, 0.36 M sucrose, 0.6 mM NaN₃, at 25 °C for 30 min. To ensure complete digestion and removal of amorphous material, the incubation mixture was removed and replaced by fresh CAF mixture four times (30 min each). Following the fifth incubation, the CAF-treated fibres were washed twice in the same solution, except that 10 mM EGTA was substituted for the CaCl₂, and no enzyme was added. Note removal of material in the Z line and appearance of filaments of uniform diameter running throughout the area occupied by the original rod bodies. Small numbers of thick filaments, originally surrounding the rods, also can be seen in scattered clumps in this section. Control experiments (not shown) did not show any morphological changes when CAF was deleted from the incubation mixture. *c*, Electron micrograph of a CAF-treated nemaline sample incubated with heavy meromyosin (HMM). An equal volume of HMM (4 mg ml⁻¹) in 67 mM potassium phosphate buffer, pH 7.0, was mixed with an aliquot of the CAF-treated fibres suspended in the EGTA washing solution and gently stirred for 1 h at 25 °C. Excess HMM was removed by four washes with EGTA washing solution. Note that the long exposed longitudinal filaments bound the HMM to form well known¹⁹ arrowhead structures (compare *b* with *c*). At present it is difficult to determine the direction of polarity of each filament, but analysis of many sections indicates that adjacent filaments often have opposite polarity. Control experiments (not shown) showed no binding of material to filaments when HMM was deleted. Bar: 0.5 μm, photographs × 16,800.

1b), samples of CAF-treated nemaline fibres were incubated with heavy meromyosin. In all cases, the exposed filaments were decorated with the heavy meromyosin forming the well known arrowhead structures similar to those obtained by decorating F-actin filaments¹⁹ (Fig. 1c).

These results show that one component of nemaline rods, the lines running the length of the rod and responsible for the periodicity parallel to the long axis of the intact structure, is composed of actin. Partially exposed filaments that resemble those exposed with CAF can also be seen following extended (up to 40 d) low ionic strength extractions¹², but CAF treatment produces more complete exposure of filaments, does not remove portions of the longitudinal filaments as does low ionic strength extraction, and is much faster. These experiments suggest the usefulness of CAF as a probe of other structures that contain actin filaments. In particular, it would be interesting to use this method to examine the nature of rod bodies and widened Z line-type structures present in various skeletal and cardiac muscle disorders²⁻⁶ to see if they, too, contain an actin backbone.

Based on comparative electron microscope studies of cross sections of Z lines and nemaline rods, MacDonald and Engel⁸ suggested that I (thin) filament profiles are continuous throughout the rods. Engel and Gomez also had suggested earlier that the longitudinal filaments may contain actin because rods disintegrate when I filaments are extracted from the fibre⁷. From our long term selective extraction experiments on nemaline muscle with use of low ionic strength buffers, we concluded that the longitudinal filaments made up the backbone of the nemaline rod, but we were unable to identify their composition clearly¹². Note that our studies reveal only the composition of the longitudinal, filamentous backbone, they do not indicate whether tropomyosin or troponin are on the filaments in the intact rods. Nor do they directly indicate the composition of filaments or material responsible for the periodic lines perpendicular to the long axis of the rods; however, because antibodies to highly purified α -actinin bind to intact rods²² and because of the specificity of CAF in removing α -actinin from Z lines¹⁸, it seems likely that at least some of this material is α -actinin. Although Sugita *et al.*²³ reported that heavy meromyosin would not bind to nemaline rods, their result is not surprising because of the large amount of dense, amorphous material present in the intact rods that is removed by treatment with CAF.

Although it has not yet been determined whether actin terminates at the edge of the Z line or makes up part of the Z line structure in normal muscle, it is interesting that actin filaments are found throughout the rods of nemaline muscle. Stromer *et al.*¹² and MacDonald and Engel⁸ have suggested the rods can be considered to represent a replicating Z lattice or lateral polymer of Z line units. Further studies on nemaline rods may be useful in helping us to understand the Z line structure.

This research was supported by the Iowa Agriculture and Home Economics Experiment Station and grants from the NIH and Iowa Heart Association. M. Y. is an MDA Fellow.

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Received 12 September; accepted 14 November, 1977.

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Does cyclic GMP mediate the slow excitatory synaptic potential in sympathetic ganglia?

THE slow excitatory postsynaptic potential (slow EPSP) of vertebrate sympathetic ganglia is elicited by the muscarinic action of acetylcholine^{1,2}. Its electrogenic mechanism appears strikingly different from that of the well-known (fast) EPSP in many respects—most notably that it is generated with no detectable increase in membrane conductance³. As this type of transmitter action is probably related to cholinergic processes in the brain^{4,5} and possibly in the spinal cord⁶, further study of the slow EPSP in the ganglia as a simpler model of the central cholinergic pathways is of great significance.

It has been proposed that cyclic guanosine monophosphate (cyclic GMP) mediates muscarinic acetylcholine action in various tissues⁷. However, to establish this hypothesis more adequately in sympathetic ganglion cells, it is crucial that the observed postsynaptic depolarisation induced by cyclic GMP⁸ be developed with membrane changes which are identical to those accompanying the slow EPSP itself. We have now used intracellular tests to show that cyclic GMP can elicit two kinds of depolarising response, one of which shows at least some similarity to the slow EPSP.

Intracellular recordings were made in the rabbit superior cervical ganglion at 34 °C, with microelectrodes filled with 1M-KCl, by methods generally the same as those already described⁹.

Extracellular application of cyclic GMP produced a steady depolarisation, but the accompanying changes in membrane resistance (r_m) were different for different ranges of concentration. (Data are all with dibutyl ester; non-butyryl cyclic GMP behaved similarly but required higher concentrations.) With lower concentrations (25 to 100 μ M) of cyclic GMP, r_m seemed somewhat decreased, with initial phase of depolarisation that reached a maximum to about 7 mV (Fig. 1, A-a). But this decrease in r_m tended to subside thereafter, whereas the depolarisation was sustained. On the other hand, when the membrane potential was clamped to the original resting level (Fig. 1, B-a) by applying steady polarising current, no decrease in r_m was observed during the action of cyclic GMP. This suggests that cyclic

GMP action itself produced essentially no decrease in r_m , and that the small decrease in r_m during the initial depolarisation of non-polarised cell might be due to a delayed rectification effect (increase in K permeability) produced by the slow depolarisation *per se*. This supposition is better tested by measuring the current-voltage relationship (I-V curve) and comparing the slope of the curves before and during cyclic GMP depolarising action (Fig. 1C). The slope of the I-V curve was found not reduced by cyclic GMP, indicating there was no decrease in r_m . When some flattening of the I-V curves were recorded in the depolarised range, there was rather slight increase in the slope with cyclic GMP. This apparent reduction of the delayed rectification seems to become more marked at 5-10 min after the onset of exposure of the ganglion to cyclic GMP. A similar increase in r_m was reported to occur in single cortical cells of the cat after the intracellular injection of cyclic GMP^{10,11}.

With approximately 10-fold higher concentrations of cyclic GMP, 500-1,000 μ M, the depolarisation was not only distinctly greater (about twofold or more), but it was now accompanied by a substantial decrease in r_m (Fig. 2A) which could not be abolished by repolarising the membrane to the

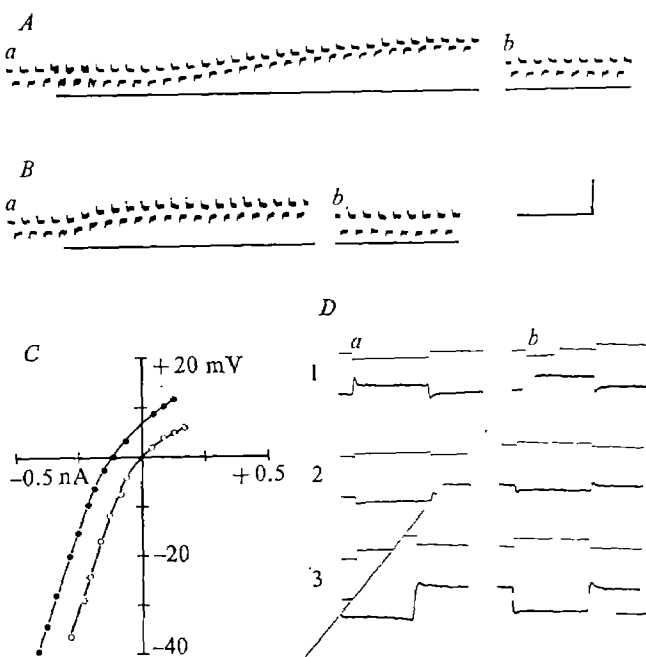


Fig. 1 Changes in membrane potential and resistance in response to lower concentrations of cyclic GMP, or to orthodromic nerve stimulation. Resting potential was -51 mV. A-a, response to stimulation at 100 μ M (horizontal bar); B-a, slow dibutyl-cyclic GMP maximal preganglionic nerve stimuli at EPSP elicited by curarine, 20 μ g ml⁻¹, was present to block 20/s (bar). (b). Membrane resistance was monitored by the fast EPSP voltage pulses resulting from the passage across size of range of constant current pulses (1 s) at a frequency of 0.5 s⁻¹ during the course of each depolarisation as seen in resting B-b; this was done by passing a steady hyperpolarising current with manual controls. Calibration: 10 mV and 10 s. C, Current-voltage (I-V) relationship obtained from the same cell in A and B, before (open circles) and 10 min after (filled circles) the application of cyclic GMP (100 μ M). A zero level of the ordinate indicates the resting potential without cyclic GMP. D, Electrotonic potentials (lower records of each pair) induced by the current pulses (upper records) of different strengths and polarity. Data correspond to the I-V curves shown in C. Pulses were passed across the membrane at the resting potential level (column a) and at the top of steady depolarisation by 100 μ M cyclic GMP (column b). Most of the cells gave 'humped' tonic responses to the passage of weak polarising currents, as seen by Blackman *et al.*¹⁸, so that the potential heights were measured at the steady plateau level when plotted in the graph in C. Calibration: 0.25 nA (hyperpolarising direction upwards) for currents and 25 mV (hyperpolarisation down) for potentials; 0.5 s time-base.

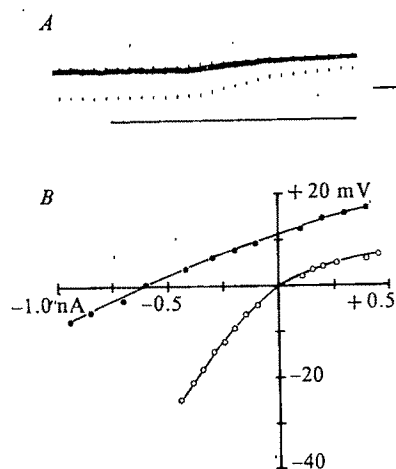


Fig. 2 Changes in membrane potential and resistance in response to higher concentration of cyclic GMP. Resting potential was -53 mV. Frequency of current pulses (200 ms duration in this case) to monitor the membrane resistance was 0.2/s. Calibration: 20 mV and 5 s. *A*, Depolarisation induced by cyclic GMP at $1,000 \mu\text{M}$; *B*, I-V relationships obtained from the same cell, with cyclic GMP (filled circles) and without (open circles).

resting potential level. Further evidence that this marked decrease in r_m is due to the action of cyclic GMP itself is seen in the altered slope of the I-V curve (Fig. 2*B*). The roughly linear portions of the I-V curves near the resting potential indicate a drop in r_m of more than 50% during cyclic GMP action. This second type of depolarising action by stronger cyclic GMP may be a more nonspecific one; it may be related to the responses with increased conductance which can be elicited by either cyclic GMP or cyclic AMP^{11,12}, and to the depolarisation of peripheral nerve by higher concentrations of cyclic GMP⁸.

The difference in r_m effects for low against high cyclic GMP is not simply a quantitatively graded one, related to the magnitude of depolarising action; although the minimum concentration to produce a detectable reduction in r_m appears somewhat variable from cell to cell with the range between 100 – $500 \mu\text{M}$, in a given cell there was always a distinct range of low concentration in which no detectable change in r_m accompanied a definite depolarisation by cyclic GMP.

During the slow EPSP depolarisation there was little or no change in r_m as reported previously³ (Fig. 1*B*). However, when the membrane potential during the slow EPSP response was temporarily repolarised to the resting level, a definite but small increase in r_m (about 20%) above resting level was usually visible (Fig. 1, *B*, but 20%) above resting level). Actually, determination of full (see also refs 3, 5, 6, 13). cell during muscarinic transmitter action reveals that (1) resistance (slope) is essentially unchanged from that seen for the control I-V curve with no muscarinic action and (2) in the depolarised range there is a substantial increase in r_m , but it only brings the slope back towards though no slope, that in the hyperpolarised range of control curve. This relationship to r_m is similar to that described for the hyperpolarised range of control curve. Thus the increase in r_m produced by muscarinic action in the depolarised range is best explained as an action antagonistic to the delayed rectification effect of depolarisation. This may be due to an action resembling the tetraethylammonium antagonism of delayed rectification¹⁵.

The postsynaptic depolarising action of a low concentration of cyclic GMP was thus found to resemble in its electrogenic nature the slow EPSP in one respect: both show no increase in membrane conductance. Indeed, if elicited in a somewhat hyperpolarised range of membrane potential

levels of -60 to -70 mV, which are probably normal ranges of resting potential of unimpaled cells, the depolarising responses to both muscarinic acetylcholine and the low concentration of cyclic GMP develop with neither increase nor decrease in membrane conductance. On this basis, there is at least some support for the hypothesis^{7,8} that the specific postsynaptic receptor for the slow EPSP response to acetylcholine operates by activating guanylate cyclase, leading to an increase in cellular cyclic GMP, (refs 16, 17, H.K. and N.S.U., unpublished) which in turn induces the subsequent depolarising response. Furthermore, this possibility of the involvement of intracellular cyclic GMP synthesis in the generation of the slow EPSP is in general agreement with our previous observation that the slow EPSP is quite selectively sensitive to the depression of oxidative metabolism^{1,3}.

This work was supported partly by a grant from the Ministry of Education of Japan, and also in part by US Public Health Service grant to one of the authors (B.L.) from the National Institute of Neurological and Communicative Disorders and Stroke.

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Received 12 May; accepted 17 November 1977.

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Postsynaptic modulation of excitatory process in sympathetic ganglia by cyclic AMP

THE principal neurones of the vertebrate sympathetic ganglia usually respond to suitable preganglionic stimuli with three types of temporally distinguishable postsynaptic potential (PSPs) in the following sequence: an initial excitatory one (fast EPSP), a slow inhibitory one (slow IPSP) and a slow excitatory one (slow EPSP)^{1,2}. These three responses can also be distinguished pharmacologically: the fast EPSP is mediated by a nicotinic acetylcholine receptor, and the slow EPSP by a muscarinic acetylcholine receptor. The synaptic pathway of the slow IPSP includes intervening adrenergic cells (called “SIF” cells³) which release a catecholamine transmitter (identified as dopamine in the case of rabbit^{4,5}), in response to the muscarinic action of acetylcholine, and in turn depolarise the ganglion cells.

It has been found that the brief application of dopamine to exhibit superior cervical ganglion produces prolonged agonism of the depolarisation induced by a muscarinic monophase can be mimicked by exogenous cyclic adenosine iontophoretically (cyclic AMP)⁷. We have now found that injected cyclic AMP into the single ganglion

cell can induce a long-lasting enhancement of the physiologically-elicited slow EPSP. Repetitive stimulation of the preganglionic nerve, in a manner shown to release dopamine from the SIF cells intraganglionically⁴ and to raise ganglionic cyclic AMP^{8,9}, was also found capable of inducing the similar change in the slow EPSP.

Isolated superior cervical ganglia of rabbit were studied at 34 °C, utilising conventional microelectrophysiological techniques¹⁰. For iontophoretic injections, the microelectrode was filled with 0.9M KCl plus 0.1M cyclic AMP (K-salt) and had tip resistance of 40 to 60M Ω . To inject cyclic AMP, 0.5nA was passed for 3 to 10 min.

Following intracellular injection of cyclic AMP, re-tests of the slow EPSP showed enhancement in 4 out of 9 cells tested. A typical example of such an effect is seen in Fig. 1. Amplitudes increased to a maximum after about 15–20 min and then decayed gradually, returning to control levels almost 2 h later. During the enhanced slow EPSPs, there were no appreciable or consistent changes in the partially curarised fast EPSPs responding to the orthodromic volleys; nor were there any significant changes in resting membrane potential or resistance. A second injection of cyclic AMP could renew the enhancement of the slow EPSP (Fig. 1). The absence of effects in some of the nine cells tested could be due partly to the marginally small amounts of cyclic AMP injected in the present method.

Control injections of current alone with a KCl-only micro-electrode in 10 other cells or more produced no such changes in the slow EPSP in the ensuing 2 h. Similarly, with electrodes containing adenosine (non-cyclic) 5'-monophosphate or adenosine triphosphate (ATP) no slow EPSP changes were observed.

A similar change in the slow EPSP could also be induced by the extracellular application of dibutyryl cyclic AMP (1 to 2.5mM added to the chamber bath for 4–8 min) in most of the ganglion cells tested. The absence of any direct hyperpolarising response to dibutyryl cyclic AMP itself, at a concentration which did produce a long-lasting enhancement of the slow

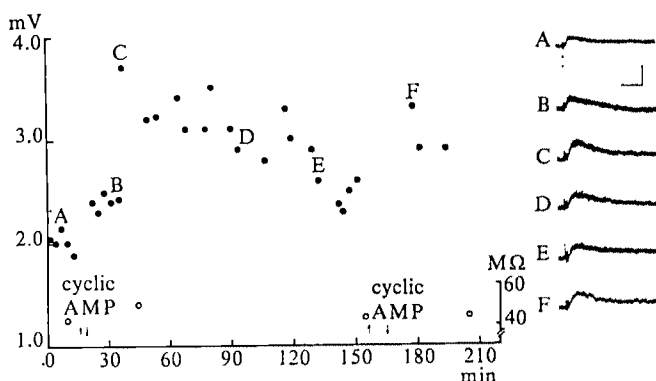


Fig. 1 Slow EPSP amplitude (filled circles) and membrane resistance (open circles) after intracellular injection of cyclic AMP. Current of 0.5nA to inject cyclic AMP iontophoretically was passed between the arrows. Each slow EPSP was evoked at 3- to 10-min intervals by a train of supramaximal preganglionic nerve stimuli (20/s for 1 s), in the presence of d-tubocurarine (20 μ g ml⁻¹) to depress the fast EPSPs. Resting membrane potential of this cell was -50mV. The cell recorded stably for more than 4 h. Inset on the right shows actual oscillograms of the slow EPSPs that correspond to the lettered points on the graph. Stimulation was given at the arrow. Calibration: 5mV and 5 s.

Membrane resistance (ordinate on right side) was measured from the plateau amplitude of the change in membrane potential produced by a constant current hyperpolarising pulse (1 s duration). All exogenous currents were passed through the recording electrode by means of a Wheatstone bridge arrangement. In this and most other experiments with cyclic AMP, the intraganglionically releasable dopamine in the whole ganglion was depleted by a pretreatment with bethanechol⁷, and therefore the slow IPSP were not seen in the oscillograms. This minimises possible effects of dopamine that could be synaptically released during the experimental period, and simplifies the interpretation of changes in the slow EPSPs.

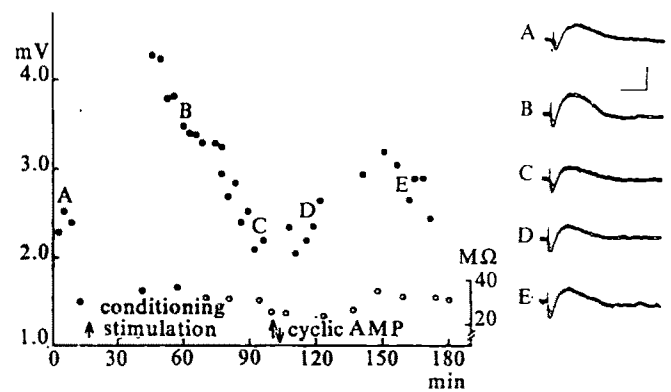


Fig. 2 Slow EPSP and membrane resistance after conditioning stimulation of preganglionic nerve (20/s supramaximal for 1 min, at the arrow) and after a later intracellular injection of cyclic AMP (between arrows). Resting membrane potential was -52mV. Otherwise as in Fig. 1, except that this ganglion was not pretreated with bethanechol, in order that synaptically releasable dopamine remains available. The latter accounts for the normally appearing slow IPSP component before the main slow EPSP in the responses shown by the oscillogram records.

EPSP, is in agreement with other studies using intracellular recordings^{11,12,19}; apparent hyperpolarising responses have been reported with the sucrose-gap technique by some¹³, but not others¹⁴.

Conditioning stimulation with repetitive preganglionic volleys (20/s for 1 min) could also be followed by a period of enhanced slow EPSPs as shown in Fig. 2. The slow EPSPs rose to more than 200% of control value at about 25 min after the conditioning, returning slowly to the original level after about 90 min. The postconditioning modulatory change also occurred without significant changes in resting membrane potential or resistance of the ganglion cell (Fig. 2). In these experiments, post-tetanic potentiation of presynaptic function could contribute to the changes, but this factor was apparently significant only during the initial 10–15 min after conditioning; this could be judged primarily from changes in the fast EPSPs, since presynaptic PTP should presumably affect both the fast and slow EPSPs similarly¹⁵. Furthermore, in some cells there happened to be no detectable slow IPSP, indicating that orthodromic volleys were ineffective in delivering functionally sufficient dopamine by release from the SIF cells⁴; in these cells the conditioning stimulation produced an increase lasting only about 15–20 min in all of the postsynaptic responses, without any longer lasting enhancement in the slow EPSP.

With surface recordings from the whole ganglion similar long-lasting modulatory effects of preganglionic conditioning were observed; in the five ganglia tested, the population slow EPSP response showed enhancement for periods of almost 3 h, with maximal increases of an average of $+60\% \pm 10.2\%$ (range $+39\% - +95\%$). Related long-lasting enhancements of muscarinically-induced postganglionic firing have also been reported to follow a conditioning train of preganglionic volleys, in superior cervical ganglia of the cat *in vivo*¹⁶, and of the rat *in vitro*¹⁷.

Our results provide further support for the proposal of Libet *et al.*⁷ that at least one of the two postsynaptic actions of dopamine, the long-lasting enhancement of the muscarinic depolarising response, may be mediated intracellularly by cyclic AMP. This effect is now demonstrable with slow EPSP responses of the single ganglion cell; it can be produced by intracellularly injected cyclic AMP, eliminating any question of an indirect action of cyclic AMP through a presynaptic release of the other transmitter(s); it is producible through the more physiological route of orthodromic nerve impulses as well as by applied dopamine or cyclic AMP. The absence of significant changes in resting membrane potential or resistance, during enhanced slow EPSP, helps to establish further the effect as an actual modulatory change in the intraneuronal mechanisms

that generate the slow EPSP response to acetylcholine. On the other hand, the absence of a direct hyperpolarising response of the single cell to extracellularly applied dibutyryl cyclic AMP, even at 2.5 mM or more (see also^{11,13}), raises a serious question about the proposed role for cyclic AMP^{13,18} as the mediator of the slow IPSP.

We thank Professors B. Libet and T. Tosaka for their help. This work was supported partly by a grant from the Ministry of Education of Japan.

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Received May 12; accepted November 17 1977.

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Ecto-ATPase deficiency in glia of seizure-prone mice

A DEFICIENCY of calcium-stimulated adenosine triphosphatase (Ca^{2+} -ATPase) is seen in brains of mice which are susceptible to audiogenic seizures¹. After studying five other animal models of idiopathic epilepsy we concluded that brain nucleotide metabolism is altered in the epileptic animals and that the lesion might not be confined to Ca^{2+} -ATPase². We speculated that the deficiency of Ca^{2+} -ATPase, which we observed in membrane-enriched brain homogenates, was the expression of incompetent brain cell ecto-ATPases. We have suggested that the genesis of seizures was related to a protracted action of translocated cytoplasmic ATP on cell membranes³ when the latter were deficient in this enzyme. We report here a significant deletion of ecto-ATPase in cultured glia cells raised from neonatal, seizure-prone mice. Between the ages of 20 to 35 d, mice of the DBA/2N strain convulse after repeated exposure to loud noise. Before and after this period, susceptibility to induced convulsions is minimal or absent. Mice of the C57 B1/6N strain are not afflicted in this manner.

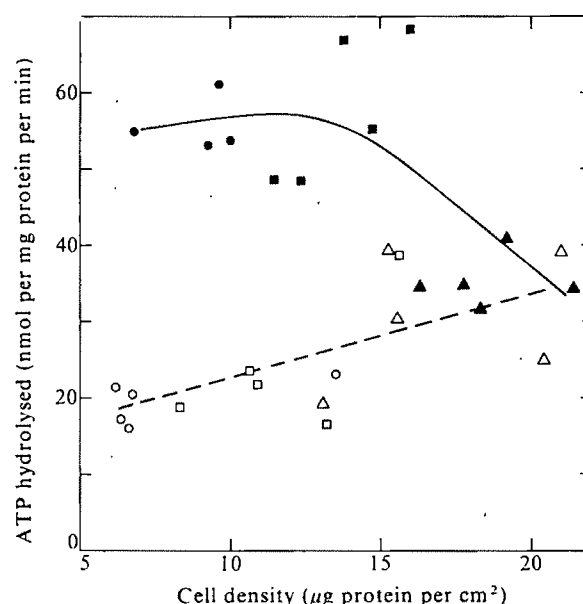


Fig. 1 Ecto-ATPase activity of normal and seizure-prone mouse glia cells. Cells of passage 4 from C57 mouse brains (solid symbols) and from DBA mouse brains (open symbols) were plated out at equal density in 9.6-cm² multi-dish tissue culture trays (Linbro). The cell monolayers were rinsed with 2 ml of modified Dulbecco's medium (mDMEM) and then incubated for 15 min with 2 mM ATP- γ -³²P in mDMEM (see Table 1). Medium was removed after incubation and the cells digested for 24 hours with 1 N NaOH. Protein in the digest was determined according to Lowry *et al.*¹⁰. Each point shown is the average of three determinations. Cultures were assayed at day 7 (●, ○), 11 (■, □) and 18 (▲, △). Curve fitting was by least squares, quadratic polynomials.

Ten separate glia cell cultures (largely of astroblast origin) were obtained from explants of newborn mouse brains according to the procedure of Shein *et al.*⁴. The cultures obtained from neonatal DBA mice (epileptic) are designated D lines and those from the C57 mice (controls), C lines. Experiments were performed on cell cultures of five different D clones and five different C clones after the lines had been propagated through three passages.

Ecto-ATPase assays were performed on intact monolayer cultures which were superfused with substrate in an iso-osmotic medium the composition of which closely resembled the culture medium. The assay of ectoenzyme activity required appropriate conditions and controls (see refs 5, 6). We also determined the activities of ecto-5'-nucleotidase, ecto-ADPase and *p*-nitrophenyl phosphatase (though it is not certain that the latter indeed is an ectoenzyme). Here, as in our earlier experiments with membrane-enriched brain fractions from DBA, C57 and C3H mice¹ these enzymes served as controls and attested to membrane integrity. The specific activity of some ectoenzymes (mol of substrate metabolised per unit time per mg of protein) can vary greatly with the cell density of cultures or with their proliferative rate⁹. Some of the enzyme assays, therefore, were

Table 1 Conditions for assay of ecto-enzymes

Enzyme	Assay medium	Substrate	Product analysed	Refs
Adenosine-triphosphatase (EC 3.6.1.3)	0.5 ml mDMEM* pH 7.4	2 mM ATP- γ - ³² P	liberated ³² PO ₄	7
Adenosine-diphosphatase (EC 3.1.3.5)	0.5 ml phosphate-free buffer, pH 7.4	1 mM ADP	PO ₄ (colorimetric)	8
5'-Nucleotidase (EC 3.1.3.5)	0.5 ml mDMEM pH 7.4	1 mM AM ³² P	liberated ³² PO ₄	7
<i>p</i> -Nitrophenyl-phosphatase (EC 3.1.3.1)	1.0 ml phosphate-free buffer pH 7.4	1 mM Tris- <i>p</i> -nitrophenylphosphate	<i>p</i> -nitrophenol (colorimetric)	5

Cells grown to confluency in 9.6-cm² multi-dish trays were rinsed with mDMEM or phosphate-free buffer⁵ before incubation. Monolayers were then incubated for 15–60 min at 37 °C and rotated horizontally at 50 r.p.m. every 90 or 180 s for 6 s.

*Modified Dulbecco's minimum essential medium; it contained neither foetal calf serum nor indicator dyes; Ca^{2+} was 0.5 mM and Mg^{2+} was 5 mM.

Table 2 Ecto-enzyme activity and growth rates of cultured glia cells from seizure-prone (DBA) and control (C57) mice

Enzyme	Day(s)	Culture density ($\mu\text{g protein cm}^{-2}$)	Specific enzyme activity* (nmol substrate hydrolysed per mg protein per min)	
			DBA	C57
Ecto-5'-nucleotidase	7	8.2 \pm 0.9	6.54 \pm 0.80 (15)	7.53 \pm 0.41 (9)
	11	12.7 \pm 0.8	5.13 \pm 0.33 (15)	5.41 \pm 0.44 (15)
	18	18.0 \pm 1.1	2.30 \pm 0.20 (15)	2.56 \pm 0.18 (9)
Ecto-ADPase	4	9.0 \pm 1.0	25.1 \pm 1.52 (15)	20.6 \pm 0.64 (15)
p-Nitrophenyl-phosphatase	4	9.0 \pm 1.0	6.30 \pm 0.26 (12)	10.41 \pm 1.37 (15)
			Growth rate†	
—			0.97 (5)	1.24 (5)
—			0.75 (5)	0.70 (5)

Culture conditions were as described in the text and in the legend to Fig. 1. Assay conditions were as for Table 1.

*Values given \pm s.e.m. with number of determinations shown in parentheses.

† $\mu\text{g protein synthesised per cm}^2$ per day.

made at different stages of confluency of the monolayer cultures. Assay conditions are given in Table 1.

When an equal number of D and C cells were plated out, the growth rates (and presumably the viability) of the clones were not significantly different. Both cultures reached about 95% confluency in 11 d. Comparison of the ecto-ATPase activity as a function of cell density revealed a large difference between the two cell lines during the proliferative phases of the cultures (Fig. 1). Ecto-ATPase activity of the glia line derived from seizure-prone DBA mice was significantly lower (Student's *t* test) than that found in C57 glia. On the 7th, 11th and 18th day of culture, D-line glia averaged 35% ($P < 0.001$), 42% ($P < 0.001$) and 86% ($P < 0.3$) respectively of the C-cell controls. During the 7–18 d culture period, ecto-ATPase activity of the C cells had decreased by approximately 35% while that of the D cells had increased nearly 55%. Therefore, on the 18th day of culture, when growth rates were markedly diminished, the ecto-ATPase activity of the epileptic glia line had reached about 85% of that of the controls. This is consistent with the observation that during the later stages of brain maturation in the DBA mouse, the susceptibility of the animal to audiogenic seizures almost vanishes. We have proposed elsewhere that the temporary insufficiency of brain Ca^{2+} -ATPase in the DBA mouse was due to the delayed expression of a more mature ATPase isoenzyme¹. We suggest that this pattern change in ecto-ATPase activity is linked to cell differentiation and (brain) organisation. Although we could not detect organisation in tissue culture, some form of differentiation (for example contact inhibition) may have evoked a similar response pattern in the experiments described here.

Table 2 summarises the values obtained for our presumptive control enzymes. No substantial differences were observed between C57 and DBA glia cells and we assume that the plasma membrane of the DBA glia was normal in other respects. The proposed involvement of glia cells in the genesis of seizures departs from the traditional concept that the epileptogenic lesion ought to be sought in the neuronal elements. Perhaps, because the role of glia in modulating neuronal excitability is poorly understood, their importance in central nervous system function has been underestimated. Several experiments have suggested, however, that neuronal excitability might be mediated by the glia, for instance, by limiting extracellular build-up of substances which might trigger synaptic transmission¹¹. A similar interpretation was made when it was found that neuroleptic drug binding increased with the enrichment of glia cell membranes in brain homogenate fractions¹². Our results do not preclude the existence of neuronal dysfunction in seizure-prone animals, but they strongly suggest that a glial abnormality is implicated in convulsive disorders.

Thus we have shown a deficiency of Ca^{2+} -ATPase, previously demonstrated in seizure-prone animals, to be amplified in the experimental system described here. The ATPase incompetency observed in membrane-enriched homogenates was associated with a lesion in the ectoenzyme activity of the plasma

membrane, and the observed metabolic error (or genetic variant) is a property of the glial elements of the brain (and possibly confined to astrocytes). Our results suggest that a key to the metabolic error in convulsive diseases may be found in the modulating action of ATP on membrane excitability thresholds and the interrelated functions of plasma membrane ectoenzymes.

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Received 16 September; accepted 21 November 1977.

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D-Glucose inhibits potassium efflux from pancreatic islet cells

ELECTROPHYSIOLOGICAL experiments have shown that D-glucose, the major physiological stimulus of insulin release, produces depolarisation and electrical activity in pancreatic beta cells¹ and that a correlation exists between these electrical changes and the stimulation of insulin release^{2–4}. Continuous recordings from single beta cells have permitted characterisation of the regular burst pattern of glucose-induced electrical activity⁵; however, the ionic mechanisms underlying these electrical changes remain unclear and both active and passive ionic fluxes may be involved^{6,7}. Islets incubated in a medium containing a very high glucose concentration (20 mM) retain more $^{86}\text{Rb}^{+}$ than do islets incubated in the presence of 3 mM glucose; this suggests⁸ that the depolarising effects of D-glucose on beta cells might be mediated, at least in part, by a decrease in K^{+} permeability. Recent experiments further showed that tetraethylammonium, a specific blocker of K^{+} conductance, potentiated the effect of glucose on both the inhibition of $^{86}\text{Rb}^{+}$ efflux and stimulation of insulin release from isolated islets⁹. The purpose of the study described here was to obtain direct evidence for an effect of glucose on potassium efflux from pancreatic islet cells and, by comparing the characteristics of this effect with the changes in insulin release and

electrical activity, gain further insight into the ionic mechanisms involved in the glucose control of the secretory function of the pancreatic beta cells. It will be shown that physiological concentrations of D-glucose, but not of L-glucose, markedly modify K^+ permeability in islet cells, that this effect requires D-

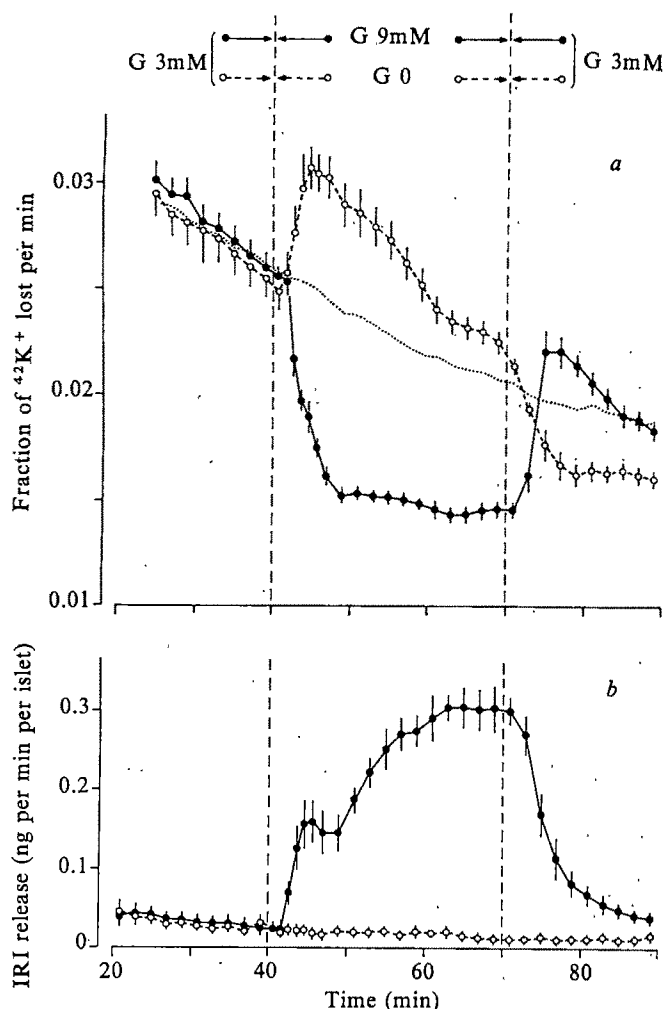


Fig. 1 Effect of D-glucose on potassium efflux (a), and insulin release (b) from perfused rat islets. The perfusate contained 3 mM D-glucose (G) except between min 40 to 70, during which D-glucose was either omitted (\circ) or raised to 9 mM (\bullet). In a, the stippled line represents control experiments in 3 mM D-glucose throughout. Values are means \pm s.e.m. of 5 experiments. Isolated islets were obtained after collagenase digestion¹¹ of the pancreas of fed male Wistar rats (275–325 g). All experiments were performed at 37 °C with a Krebs–Ringer bicarbonate solution, pH 7.4, containing 6 mM K^+ , supplemented with 0.5% (w/v) bovine serum albumin and equilibrated with $O_2:CO_2$ (94:6). The perfusion system was similar to that previously described¹⁰, but with a smaller chamber (0.3 ml) and a reduced flow rate (1.1 ml min⁻¹). The dead time of the system is 1 min, but no correction was made in the presentation of the results. Effluent fractions were collected at 2-min intervals except for 1-min intervals from min 40 to 46. In efflux experiments, groups of 75–100 islets were first incubated at 37 °C for 150 min, in 1 ml medium containing 3 mM D-glucose and 6 mM ^{42}KCl (IRE, Fleurus, Belgium; 3.5–6.5 mCi mmol⁻¹). They were then washed three times with 5 ml nonradioactive medium at room temperature and transferred to the perfusion chambers. $^{42}K^+$ in the effluent fractions and remaining in the islets at the end of the experiments was counted in a liquid scintillation spectrometer by the Cerenkov radiation, after addition to each sample of 10 ml of a 3-mM aqueous solution of the wavelength shifter 7-amino-1, 3-naphthalene-disulphonic acid¹². After correction for decay, the fractional efflux of $^{42}K^+$ released during the time interval/ $^{42}K^+$ remaining in tissue during that time interval) was calculated for each collection interval. In release experiments, groups of 15 islets were first incubated at 37 °C for 150 min in 1 ml medium containing 3 mM D-glucose and then transferred into the perfusion chambers. Immuno-reactive insulin (IRI) was measured in the effluent fractions by a double antibody method¹³ with rat insulin as standard.

glucose phosphorylation but is not the consequence of insulin release and, finally, that this change in K^+ permeability may account for the initial depolarising effect of the sugar on beta cells.

A perfusion system^{9,10} was used to monitor continuously the dynamics of $^{42}K^+$ efflux from preloaded isolated rat islets. At concentrations of D-glucose below 5 mM, the efflux of $^{42}K^+$ did not follow simple exponential kinetics, as illustrated by the progressive decrease in the $^{42}K^+$ fractional efflux recorded in the continuous presence of 3 mM D-glucose (Fig. 1a). Withdrawal and subsequent reintroduction of the sugar were followed by prompt augmentation and reduction in the efflux rate. An increase of D-glucose to 9 mM markedly diminished and also stabilised the fractional rate of $^{42}K^+$ efflux (Fig. 1a); this effect of high D-glucose was completely reversible on return to the lower concentration of 3 mM. The concomitant changes in insulin release are shown in Fig. 1b. Omission of glucose did not change the basal rate of secretion, whereas stimulation with 9 mM D-glucose elicited the typical biphasic increase in insulin secretion. It should be noted that the first changes in efflux rate and in insulin release were recorded at the same time (3 min after the switch of the stopcock), but that only the changes in release exhibited biphasic kinetics.

The specificity of this effect of D-glucose was tested by comparison with its non-insulinotropic, non-metabolised¹⁴ stereoisomer L-glucose, which does not produce electrical changes in beta cells⁷. In three experiments (not shown), 6 mM L-glucose was added to a perfusate containing 3 mM D-glucose without significantly changing the rate of $^{42}K^+$ efflux as compared with controls maintained in 3 mM D-glucose alone.

The requirement of extracellular calcium for glucose stimulation of insulin release has long been established¹⁵. Figure 2

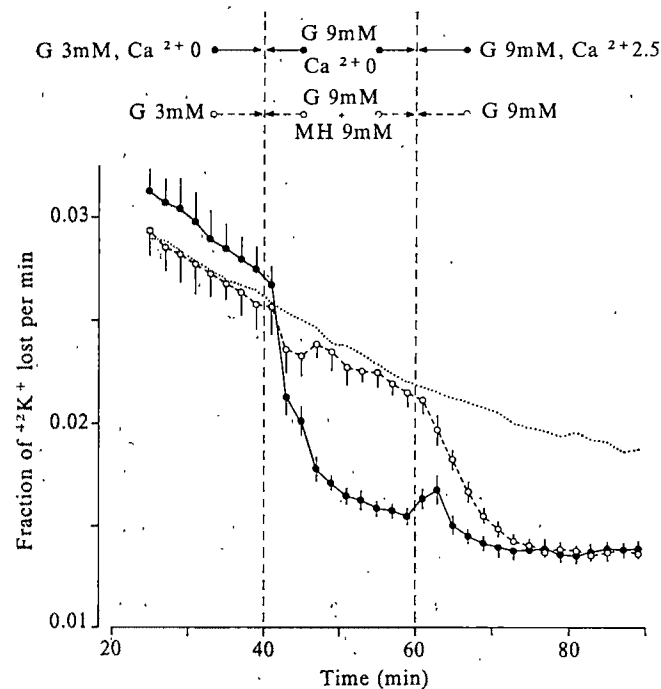


Fig. 2 Effects of the absence of calcium (\bullet) or of the presence of mannoheptulose (MH) (\circ) on D-glucose induced inhibition of $^{42}K^+$ efflux from perfused rat islets. After incubation with $^{42}K^+$ as described in the legend to Fig. 1, the islets were transferred to the perfusion chambers and effluent fractions were collected at 2-min intervals. D-glucose concentration of the perfusate was 3 mM from the start of the efflux and raised to 9 mM from min 40 onwards. In one series of experiments (\bullet), Ca^{2+} was omitted from the medium from min 0 to 60 (total $Ca^{2+} < 25 \mu M$) and added to the medium (2.5 mM) from min 60 to 90. In the other series of experiments (\circ), 9 mM mannoheptulose was added to the perfusate during the first 20 min of stimulation with 9 mM D-glucose (min 40 to 60). The stippled line represents control experiments in the presence of 2.5 mM Ca^{2+} and 3 mM D-glucose throughout. Values are means \pm s.e.m. of four experiments.

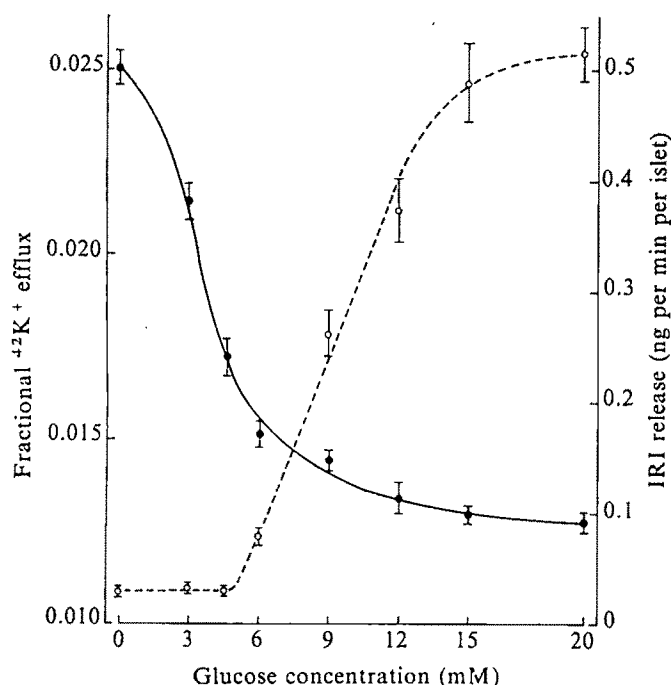


Fig. 3 Effect of different D-glucose concentrations on $^{42}\text{K}^+$ fractional efflux (●) and immunoreactive insulin release (○) from perfused rat islets. After incubation with $^{42}\text{K}^+$ as described in the legend to Fig. 1, the islets were transferred to the perfusion chambers. The fractional release of $^{42}\text{K}^+$ was measured in identical conditions at all concentrations of D-glucose. Each experiment consisted of an initial period of 36 min in the presence of 3 mM D-glucose and a final period of 28 min in the presence of only one of the various glucose concentrations studied. Effluent fractions were collected at 4-min intervals and the fractional release of $^{42}\text{K}^+$ for individual experiments was defined as the average value for the last 16 min of efflux. Values are means \pm s.e.m. of four experiments. The rate of insulin (IRI) release was studied in a similar manner except that in each experiment four alternate D-glucose concentrations were studied in sequence (starting with the lowest concentration). Values are means \pm s.e.m. of six experiments.

shows that the absence of calcium in the medium did not prevent 9 mM D-glucose from reducing the fractional efflux of $^{42}\text{K}^+$ from preloaded islets and thus demonstrates that the changes in efflux are not a mere consequence of the changes in release. Calcium introduction at 9 mM D-glucose elicited a small and very transient increase in K^+ efflux.

Mannoheptulose, which inhibits glucose phosphorylation in pancreatic islets and glucose stimulation of insulin release¹⁴, reversibly prevented the D-glucose-induced reduction in K^+ efflux (Fig. 2). This observation may indicate that glucose has to be metabolised by islet cells to reduce their permeability to K^+ and is in keeping with the report⁷ that mannoheptulose prevents glucose-induced electrical changes in beta cells.

The relationship between D-glucose concentration and the fractional efflux of $^{42}\text{K}^+$ or the rate of insulin release is shown in Fig. 3. The dose-response relationship for secretion displayed the well established sigmoid shape, with a threshold between 4.5 and 6 mM and a half-maximal response around 9 mM D-glucose. In contrast, the major reduction in K^+ efflux rate occurred at low glucose concentrations, below the threshold for stimulation of release. Above 6 mM D-glucose, only minor further changes in efflux rate were recorded, whereas the rate of release increased considerably. The similarity of the glucose dependency curves of glucose metabolism and insulin release has often been reported¹⁴. This does not necessarily imply that the important changes in K^+ permeability observed at the low glucose concentrations are unrelated to glucose metabolism. Thus, the changes in the overall glucose metabolism may not be exactly representative of the changes occurring at all the successive steps of its metabolism; furthermore, it has been recently reported that glucose utilisation and lactate formation

by islets perfused in a system analogous to that used in this study markedly increased at glucose levels which do not stimulate insulin release¹⁵.

Although K^+ efflux was measured from a heterogeneous population of cells, of which beta cells represent only two-thirds, an instructive comparison can be made with electrical recordings obtained directly in beta cells. When glucose is increased to a stimulatory concentration, the membrane potential first decreases and then starts to oscillate in slow waves (bursts) between a depolarised plateau, on to which fast spikes are superimposed, and a more polarised silent level⁶. The duration of the bursts and the frequency of the spikes are both strongly correlated with insulin release and exhibit the same sigmoidal increase as a function of glucose concentration, with a threshold at 5 to 6 mM⁵. In contrast, the membrane potential between the bursts is glucose independent above that threshold. That K^+ permeability diminishes most markedly for changes between 0 and 6 mM D-glucose suggests that the depolarisation between the resting membrane potential and the threshold potential⁵ at which the burst activity (and insulin release) starts is due to the reduction of the beta cell membrane permeability for K^+ . Once the beta cells are sufficiently depolarised, a Ca^{2+} system (as suggested by disappearance of burst and spike activity after Ca^{2+} removal or addition of the Ca^{2+} antagonist, D 600)^{4,7,17} can be activated. The repolarisation at the end of each burst has, tentatively, been explained either by inactivation of the Ca^{2+} transport system or by activation of an electrogenic Na pump^{6,7}. It is also possible that the duration of the bursts (and the magnitude of the concomitant insulin release) is partially controlled by the K^+ permeability of beta cells. This is supported by the further, although small, reduction in K^+ efflux at suprathreshold concentrations of glucose and by the potentiation of insulin release by tetraethylammonium⁶.

I thank M. Nenquin for assistance, S. Vanhemelryck for editorial help and Professors D. P. Cameron, R. Casteels and A. E. Lambert for reading the manuscript. This work was supported by a grant from the FRSM, Brussels.

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Received 18 August; accepted 15 November 1977.

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Distribution of Na^+ pump sites in the frog gallbladder

THE energy-dependent transport of fluid across epithelia has been best explained by the three-compartment conception of Curran and MacIntosh¹. This model couples the active 'pumping' of solute with the elaboration of an absorbate of specific tonicity by postulation of local osmosis within the epithelium. This reasoning has been extended in the 'standing-gradient' hypothesis², which accounts for isotonic or hypertonic fluid transport by means of epithelial geometry and the specific transport properties of the cell mem-



Fig. 1 Radioautograph of the frog gallbladder exposed to 1 μ M 3 H-ouabain for one hour followed by a 30-min wash period in cold, ouabain-free Ringer's solution. Grains, indicating 3 H-ouabain binding sites, are distributed on the lateral membranes along the whole length of the intercellular space. Note the large number of grains located at the base of the epithelial cells, especially in comparison to the number located near the junctional region between the cells ($\times 1,440$).

brane. The hypothesis depends upon a quantitative assessment of several parameters (membrane hydraulic conductivity, solute-pump distribution and dimensions of epithelial intercellular spaces)³. For example, the model best explains isotonic reabsorption by the gallbladder when it is assumed that the solute-input region (the site of energy-dependent Na^+ pumping) is confined to the apical portion of the lateral intercellular space². To test this important assumption, we studied the distribution of Na^+ pumps in frog gallbladders with radioautography of 3 H-ouabain binding sites³. This is a specific procedure for identifying the site of the enzyme, $\text{Na}^+ \text{--} \text{K}^+$ ATPase^{6,7}, which in this tissue⁴, as well as others, is equated with the Na^+ pump. We find that Na^+ pumps are present only on the basal-lateral surface of the epithelial cells, consistent with their providing solute to the intercellular spaces as the site for local osmosis. However, since the pumps are not limited to the apical portion of the intercellular space, the results do not support the 'standing-gradient' view of isotonic fluid transport.

Gallbladders of the bullfrog, *Rana catesbeiana*, were

dissected free and washed in Na^+ -Ringer's solution⁸ to remove the bile salts. Then the bladders were divided into small pieces (approximately 5–10 mg) and incubated in Ringer's solution in a shaking water bath at room temperature. 3 H-ouabain was added at a final concentration of 1 μ M and the incubation continued for one hour. This concentration was chosen because it is below the level at which nonspecific binding becomes a major factor⁸. At the end of the incubation period, the vials containing the tissue were drained and refilled with ice cold, ouabain-free Ringer's solution and placed in an ice bath. The medium was changed twice more at 10-min intervals and then the tissue was either quickly blotted and placed in previously weighed vials for wet weight determination or frozen in liquid freon cooled by liquid nitrogen. Frozen pieces of bladder were freeze-dried, fixed and embedded as previously described⁷ except that the tissue was exposed to osmium vapours for three hours to ensure adequate fixation. Sections (1 μ m) were dried on glass slides, dipped in NTB-2 emulsion (Eastman Kodak) and exposed for 1–6 weeks at 4 $^\circ\text{C}$. Quantitative analysis of the grain distribution in each gallbladder was accomplished by a computer-assisted routine similar to the techniques of Mills *et al.*⁷. Hard copies of radioautographs were taped onto a 'spark-pen' data tablet which was serially interfaced to a PDP 11/10 computer and a Princeton 801 graphics terminal. Positional information (the *x-y* coordinates of field boundaries and grains) was input to the terminal and the computer when the spark-pen was touched to the hard copy overlying the sensitised screen of the data tablet. For each field counted, the epithelial thickness was divided into 10 equal slices beginning at the mucosal border and ending at the basal lamina. In this way, the location of each grain was recorded as its relationship to each slice. The final data were then reported as a histogram of the frequency of grains in each slice.

Figure 1 is a radioautograph of a gallbladder exposed to 3 H-ouabain. Exposed silver grains representing 3 H-ouabain binding sites are associated with the entire length of the intercellular space. The most striking observation is that there is no concentration of grains at the apical end of the intercellular space, as might be predicted, while a large number of grains are preferentially located in the basal one-third of the epithelium. A summary of the grain distribution in gallbladders treated as in Fig. 1 is shown in Table 1. The frequency of grains is fairly uniform for slices 1–7. Slices 8, 9 and 10 show a stepwise increase in the number of grains, with slice 10 having twice as many grains as would be expected for an even distribution.

The observed distribution of grains, representing Na^+ pump sites, may have been due to the inability of 3 H-ouabain to reach the apical end of the intercellular space. This is probably not the case, as increasing the incubation time to two hours, or raising the concentration to 5 μ M, had no apparent effect on the distribution of binding sites. In addition, uptake measurements showed that ^{14}C -inulin reached equilibrium in 10 min, while 3 H-ouabain reached an equilibrium binding value in 15 min. Therefore, the localisation of grains along the lateral intercellular space of the gallbladder is presumed to reflect accurately the distribution of Na^+ pump sites along the whole length of the plasma membrane bordering the space. The concentration of pump

Table 1 Distribution of 3 H-ouabain binding sites in the frog gallbladder

Slices									
1	2	3	4	5	6	7	8	9	10
6.20	7.10	7.23	6.95	7.59	8.05	9.05	12.01	16.10	20.00
± 0.84	± 0.76	± 0.34	± 0.91	± 0.92	± 0.87	± 0.52	± 1.25	± 2.55	± 1.30

Distribution is represented as the percentage of grains (\pm s.e.m.) in each of 10 slices of equal cross-sectional area. Slice 1 begins at the mucosal border and slice 10 ends at the basal lamina. All values corrected for background. (*n* = 5).

sites at the basal end of the cell may be due to the membrane amplification which occurs there⁸; the distribution of pumps may, in fact, be uniform with respect to membrane surface area.

The standing gradient theory would be favoured by a pump distribution which restricted solute input to the apical portion of the intercellular space (the 'dead' end of the channel)¹². Our results, however, indicate that the solute input region is distributed along the entire length of the lateral intercellular space of the gallbladder. This observation, as anticipated and discussed by Diamond and Bossert², does not disprove the standing-gradient hypothesis, but requires the use of extreme values for some of the other important parameters in the model. Isotonicity could still be achieved with a uniform pump distribution if the water permeability of the plasma membranes lining the space was increased upwards. Hill¹⁰, however, has argued that even if the solute pumping region is confined to the upper 10% of the channel length, the osmotic permeability would have to be 3 to 4 orders of magnitude greater than that which has been measured in order to predict an isotonic absorbate by the Segel equation¹¹ for the standing-gradient model. Thus, while the localisation of Na⁺ pumps in the frog gallbladder, as reported here, does not disprove the standing-gradient osmotic flow model, it contributes significantly to the growing body of thought¹² that an alternative explanation for the tonicity of absorbed fluids must be sought.

We thank Mr Quincy Moy and Ms Bonnie A. P. Lord for technical assistance. This research was supported by USPHS grants HE 06664 and AM 17372. J. W. Mills was a fellow of the Pharmaceutical Manufacturer's Association Foundation during this work.

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Received 12 July; accepted 18 November 1977.

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Sequences and efficiencies of proposed mRNA terminators

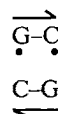
THE base sequences at the 3' end of the different messenger RNA molecules show a pattern of a GC-rich region followed by five to eight Us (refs 1,2). Gilbert's hypothesis¹ that the stability of the template duplex DNA (or of the mRNA–DNA hybrid, or both) determines termination of transcription, implies that the DNA termination sequences, and thus the mRNA sequences at the 3' end, are not unique. Rather, the DNA sequence of the template presumably expresses a certain structural stability, and this pattern of stabilities is responsible for the termination of

transcription. Using these ideas we have found five (but only five) sequences in Φ X174 DNA which have a common pattern of stabilities with previous terminator sequences. These sequences are proposed to be responsible for termination of transcription in Φ X174. Three of the five sequences have five or six As following a GC region instead of the Ts present in known terminators. The lengths of mRNAs predicted on this basis are in excellent agreement with published observations; however, sequence determination of the messengers will be necessary to test our proposal rigorously.

We have analysed those termination sequences given by Gilbert in terms of free energy of formation of each of the Watson–Crick nearest-neighbour sequences. There are some data available from thermodynamic studies of synthetic deoxypolynucleotides³, but we have used the complete set of free energy values determined for RNA double strands⁴. The relative stabilities are

GG > GC > CG > AG ~ TG ~ AC ~ TC > AT ~ TA > AA

For simplicity in representing the sequence in these terms, the nearest neighbours are labelled from 1 to 6 in order of decreasing stability. Note that these numbers characterise a sequence of two base pairs, not a single base pair. For example, GC represents the sequence



The known termination sequences^{1,2} are characterised by a most stable nearest neighbour (GG) followed by two variable terms, then a series of weak nearest neighbours such that at least five of seven of the subsequent terms are 6 on the stability scale. Known termination sequences contain oligo U sequences, but we propose that oligo A sequences are also possible; it is the stability of the oligo dA–dT region which is important.

An analysis of the sequence of the plus strand of Φ X174 DNA (ref. 5) reveals only five locations where this pattern of nearest neighbour stabilities occurs (Fig. 1). There are three promoters^{6,7} *in vitro* and the exact location of these has been determined by Sanger⁵. Using these three known mRNA initiation sites and our five proposed termination sequences, the expected mRNAs may be listed according to their molecular weights and which protein codes they contain. A comparison between this prediction and the mRNA found *in vivo*⁸ is given in Table 1. In general, excellent agreement is found.

Hayashi *et al.*⁸ separated fourteen peaks of different molecular weight mRNAs by electrophoresis. By hybridisation of these fractions with restriction fragments, those authors were able to orient nine mRNA fractions on the Φ X174 genome. All were accounted for by predicted mRNAs; seven showed exact agreement in both size and content (fractions 0 and IX are less certain).

Hayashi *et al.*⁸ reported that RNAs starting at promoter A' are rapidly degraded *in vivo*, with the possible exception of RNA species I. In Table 1, the predicted RNAs which do not have counterparts *in vivo* are those that initiate at promoter A'; and the observed fractions *in vivo* which do not correspond to predicted messengers are of smaller molecular weight and thus may represent degradation products.

Of particular significance is the observation that for each of four predicted terminators, one or more observed mRNA fractions may be assigned. No additional terminators need be proposed to explain the observed data. None of the predicted mRNAs which are terminated by T₁ is observed experimentally; therefore, they are not shown in Table 1. Since T₁ differs from the other predicted terminators by the presence of a guanine residue interrupting the AT-rich region, and thus contributing two stronger stability terms, it may be expected to be a less efficient terminator. Axelrod⁷, however, has observed a rho-dependent terminator *in vitro* which maps at a location in agreement with our predicted T₁.

Terminator [*]	Position [†]	Sequence and Stability Terms	
T ₄	3961	G G A G G C	T T T T T T A T G G T T
		1 4 4 1 2 4	6 6 6 6 5 5 4 1 4 6
T _H	2635	C G A C C C	T A A A T T T T T T C C
		3 4 4 1 1 4	5 6 6 5 6 6 6 6 4 1
T ₂	854	A A A G G T	A A A A A C G T T C T
		6 6 4 1 4 5	6 6 6 6 4 3 4 6 4 4
T _G	2308	A G C G G T	A A A A T T T T A A T
		4 2 3 1 4 5	6 6 6 6 5 6 6 6 5 6 5
T ₁	4429	T A C C C C	A A A A G A A A G G T
		5 4 1 1 1 4	6 6 6 6 4 4 6 6 4 1 4

Fig. 1 Proposed terminator sequences and stability terms for ΦX174. The approximate location of the last residue transcribed is before the right-hand solid line, except for terminator T_H (indicated by the dashed line).
* Notation from ref. 7; T_G and T_H refer to terminators labelled "start of gene G" and "start of gene H", respectively. † Indicates location of first base listed, using numbering of ref. 5.

Since Hayashi *et al.*⁸ have given values for the relative number of molecules of each of the fractions mapped, the efficiencies of each predicted terminator may be calculated. This calculation uses only those fractions assigned to starts at promoter A. The results in Table 2 are consistent with Axelrod's *in vitro* observations of their primary sequence. The terminator sequences are listed in order of decreasing efficiency in Fig. 1. All terminators require a strong nearest-neighbour interaction, outlined in the box at the left. The other boxed region is composed of ten nucleotides. The efficiency of termination seems to be correlated with the number

Table 1 Comparison of mRNAs found by prediction and by experiment					
<i>In vivo</i> [*]			Prediction		
Fraction	Mol. wt × 10 ⁻⁶	Proteins	Mol. wt × 10 ⁻⁶	Proteins	Promoter/ Terminator [†]
0	2.3	(C) DEFGHABCDE	2.61 2.29 2.00	ABCDEJFGHABCDE BCDEJFGHABCDE DEJFGHABCDE	P _A /T ₂ P _A /T ₂ P _G /T ₂
I	1.8	ABCDEFGH	1.83	ABCDEJFGH	P _A /T ₄
II	1.5	BCDEFGH	1.52 1.38 1.27	BCDEJFGH ABCDEJF(G) ABCDEJF	P _A /T ₄ P _A /T _H P _A /T _G
III	1.3	(C)DEFGH	1.23	DEJFGH	P _G /T ₄
IV	1.1	BCDEFG	1.07	BCDEJF(G)	P _A /T _H
V	0.98	BCDEF	0.96	BCDEJF	P _A /T _G
VI	0.82	(C)DEF	0.79 0.78	DEJF(G) ABCDE	P _G /T _H P _A /T ₂
VII	0.73	?			
VIII	0.66	?	0.67	DEJF	P _G /T _G
IX	0.6	BCDE	0.46	BCDE	P _A /T ₂
X	0.52	?			
XI	0.43	?			
XII	0.32	?			
XIII	0.23	D(E)	0.17	DE	P _G /T ₂

^{*}From ref. 8.
[†]Notation as in ref. 7.

vation that T₄ is a strong terminator, whereas T₂ is less effective. In addition, the terminator designated T_H is seen to have a similar efficiency to T₂, and T_G is even less effective.
The relative efficiencies of the terminators may be related to

of adjacent T or A residues and their distance from the strong nearest-neighbour interaction. Thus, T₆ starting in the first position outlined is more efficient than A₆ in the same place or T₆ starting further to the right. All of these are more efficient than A₅

Table 2 Efficiency of terminators					
Promoter	Terminator	Fraction	Relative no. of molecules	Terminator efficiency	Sequence
P _A	T ₂	0	0.3	—	GGCTTTTTTATGG
P _A	T ₄	II	2.2	79	CCCTAAATTTTTT
P _A	T _H	IV	3.1	53	GGTAAAAAACGTT
P _A	T ₂	IX	9.5	50	GGTAAAAATTTTA
P _A	T _G	V	3.0	34	
Relative no. of molecules					
			Observed	Predicted	
P _G	T ₂	0	0.3	0.3	
P _G	T ₄	III	2.3	2.1	
P _G	T _H	VI	5.2	2.9	
P _G	T ₂	XIII	9.0	(9.0)	
P _G	T _G	—	—	2.8	

starting in the first position. Given our assumptions about the importance of weak nearest-neighbour interactions in this region, it seems reasonable to speculate that the presence of G after A₅ in T₁ may make this a much weaker, or totally ineffective, terminator. Yet in view of the *in vitro* evidence, this may act effectively in the presence of rho factor. There is obviously still much to be learned about terminator efficiency and sequence, but here we stress the importance of duplex stability (DNA-DNA and DNA-RNA).

On the basis of terminator efficiencies calculated from promoter A, the relative number of molecules for each terminator for another promoter can be calculated, given the assignment of any one mRNA for that promoter. Thus for promoter G, the distribution of RNAs may be calculated given the assignment of RNA XIII to T₂. Good agreement is found for fraction O and III, but one finds that fraction VI contains nearly twice as many molecules as expected. This may be explained by reference to Table 1, where it is seen that two mRNAs of the same molecular weight, but arising from different promoters and terminators, have probably been collected together as fraction VI. Determination of the base sequences of the mRNAs can verify the proposed terminators.

This work was supported in part by National Institutes of Health and by the Division of Biomedical and Environmental Research of the United States Energy Research and Development Administration.

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Received 11 July; accepted 14 November 1977.

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Direct proton exchange between complementary nucleic acid bases

SPECIFIC interaction between complementary nucleic acid bases, in which adenine forms strong hydrogen bonds specifically with thymine (uracil) and guanine with cytosine, is believed to be the molecular basis for the transfer of genetic information. Infrared spectroscopy and nuclear magnetic resonance have shown that such specific interaction is intrinsic to nucleic acid bases¹⁻⁵. In spite of much knowledge about the static structure of the interaction⁶⁻⁹, little is known about the dynamic property of the base pairs. We report here quantitative evidence of direct proton exchange between complementary nucleic acid base derivatives.

9-Ethyladenine (9EA) and 1-cyclohexyl-5-bromouracil (5BrU) can form dimers in chloroform solution¹⁰. Proton magnetic resonance spectra of a 9EA-5BrU (1:1) mixture are shown in Fig. 1. When the amino proton signal of 9EA was irradiated, the imino proton signal of 5BrU disappeared. Similarly irradiation at the imino proton of 5BrU reduced

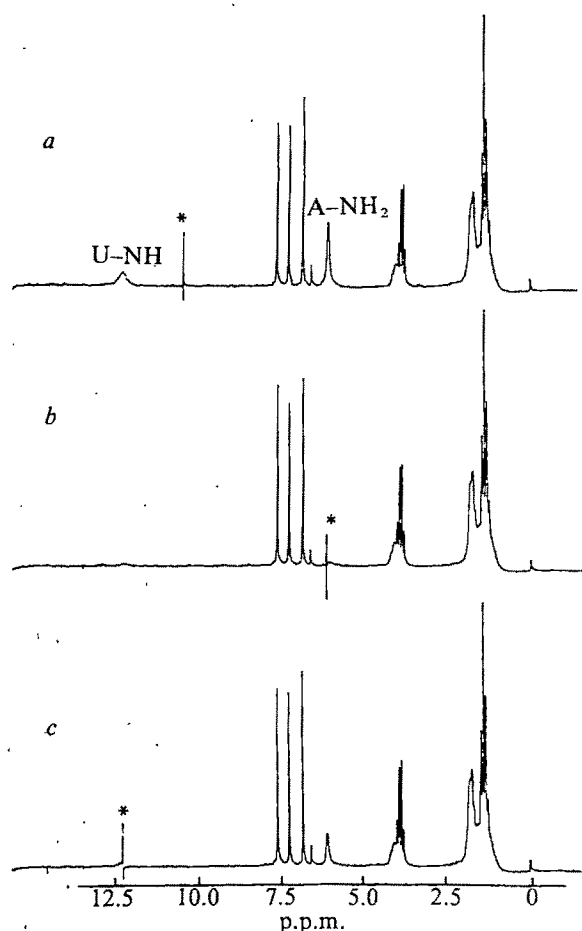


Fig. 1 ¹H NMR spectra of a mixture of 9-ethyladenine and 1-cyclohexyl-5-bromouracil (both at 0.2 M) in chloroform. *a*, Irradiated at the position where is no signal. *b*, Irradiated at the amino proton of 9-ethyladenine. *c*, Irradiated at the imino proton of 1-cyclohexyl-5-bromouracil. Asterisk indicates the irradiated position.

the intensity of the amino proton of 9EA. With dimethylsulphoxide (DMSO) solution of the 9EA-5BrU (1:1) mixture, there was a smaller decrease in peak intensity than with chloroform solution. DMSO more effectively disturbs the formation of solute-solute hydrogen bonds. Thus the observed decrease of the nonirradiated proton signal correlates closely with the quantity of hydrogen bonded dimers between 9EA and 5BrU.

These results can be explained as follows. Protons are reversibly exchanging between the amino group of 9EA and the imino group of 5BrU, and thus the irradiated proton moves to the nonirradiated site through the formation of hydrogen bonded dimers. The exchange of saturated protons produces partial saturation of the nonirradiated proton site to weaken the intensity of the nonirradiated proton resonance, if the exchange rate is faster than the relaxation time^{10,11}. Irradiation at the signal of water proton contaminated in solution gives little change in the intensity of other proton resonances. This suggests that most protons do not exchange by way of the water in the solvents.

The same experiment was performed using a mixture of 9-ethylguanine (9EG) and 1-methylcytosine (1MC) in DMSO. In this system it is interesting that proton exchange occurs between the imino proton of 9EG and the amino proton of 1MC (Fig. 2), but the amino proton of 9EG does not exchange with both the amino proton of 1MC and the imino proton of 9EG (Table 1). If the formation of cyclic hydrogen bonds of the G-C pair^{8,9} is a sufficient condition for the exchange of protons, the amino proton of 9EG should ex-

Table 1 Partial saturation induced by intermolecular exchange of saturated protons

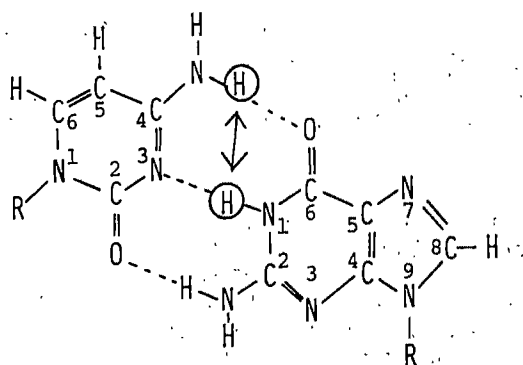
Base pairs	Solvent	Irradiated nuclei	Decrease in the peak intensity (%)			
			G-amino	C-amino	A-amino	U-imino
9-ethyladenine + 1-cyclohexyl-5-bromouracil*	CDCl ₃	A-amino U-imino H ₂ O			36 0	100 0
	DMSO	A-amino U-imino H ₂ O			17 0	40 0
9-ethylguanine + 1-methylcytosine†	DMSO	G-amino		12		20
		C-amino	12			86
		G-imino	0	71		
		H ₂ O	3	5		20

*, 0.2 M.

†, 0.1 M.

change with the other protons participating in hydrogen bonds, in view of the distance between the protons. These results make us consider factors other than distance for the mechanism of intermolecular proton exchange. The proton transfer induced by the imino and enol tautomers¹² or protonated species¹³ can be considered as possible explanations.

For this we shall estimate quantitatively the life time of the proton exchange. We consider the system where the nucleus X exchanges between site A and site B, and the nucleus X in the site B is saturated by irradiation at the A site proton. Forsén and Hoffman's analysis^{9,10} gives 0.1 s as the life time for the proton exchange if we use the data of the spin lattice relaxation times observed separately (0.23 s for A-NH₂). Thus the proton exchanges 10 times in a second for the 9EA-5BrU system in chloroform.

**Fig. 2** Exchangeable protons in the G-C pair.

Here we have used model compounds for nucleosides, and our experiments were carried out only for the monomer systems. In spite of these limitations, there is no reason to deny proton exchange in nucleic acid. The structures of nucleic acid bases which are realised at the time of the proton exchange are different from the normal structures. Although we do not know the real effect of proton exchange in complementary base pairs on biological process, such altered structures might induce mispairing in the replication and transcription of nucleic acids¹⁴.

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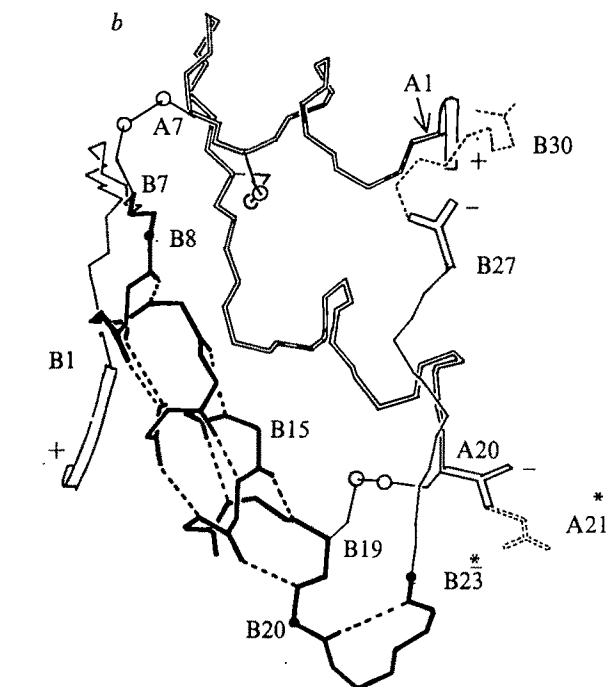
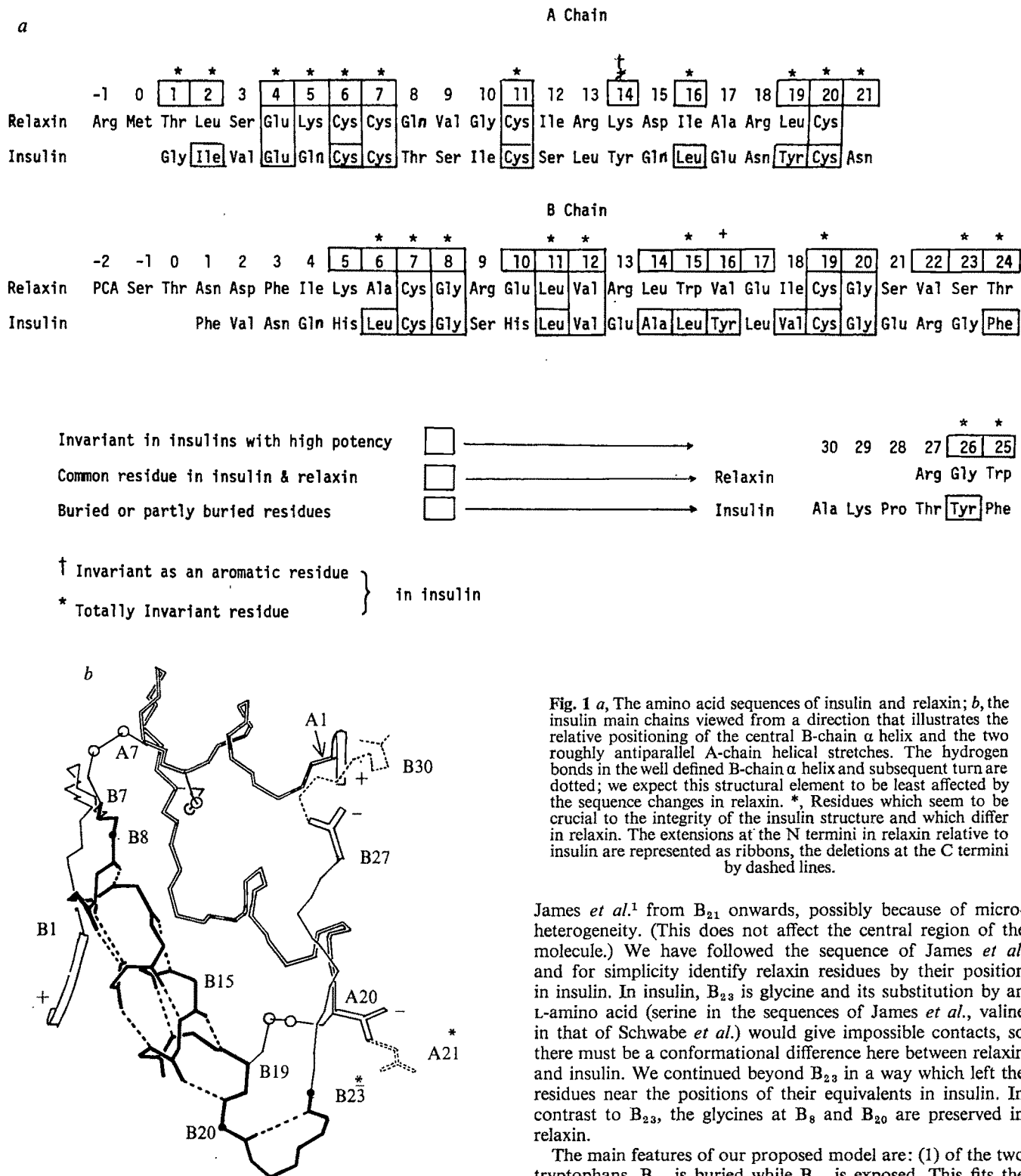
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Relaxin and its structural relationship to insulin

THE sequence of the ovarian peptide, relaxin, has recently been reported^{1,2} and the observation¹ that it can be accommodated into the insulin fold has been discussed^{3,4}. Only 11 residues, including the cystines, are common to insulin and relaxin (Fig. 1), but the probable identity of the cystine pairings and the preservation of the hydrophobic character of buried residues suggests some structural homology between the two hormones. The extent of sequence changes and the remote relationship between the corpus luteum and the pancreas, the respective sources of relaxin and insulin, makes homology of the two hormones most interesting. We have therefore rigorously examined the relaxin conformation by a computer graphics system and found it possible to accommodate the relaxin sequence within the insulin main-chain geometry.

We first considered the packing of residues whose equivalents in insulin are buried or partly buried. We chose molecule 2 of the 2-zinc insulin⁵ structure for the comparison since the conformations in this monomer are most closely related to those of 4-zinc pig insulin⁶ and the dimeric hagfish insulin (Cutfield, J. F. *et al.*, in preparation). The atomic positions of this insulin molecule are now accurately known and for the buried residues the estimated errors are 0.1-0.2 Å. Using Labquip models, we found that the internal hydrophobic residues of insulin could be replaced by the equivalent relaxin residues without the apparent need to adjust the main-chain conformation. In particular, there was an extensive cavity into which tryptophan B₁₅ seemed to fit nicely. Because of the limitations and inaccuracies in model building which we consider might be misleading, we then used a computer graphics system^{7,8} to superimpose the relaxin chains on to the insulin backbone. Any short contacts were indicated immediately by dotted lines between the offending atoms. Side-chain conformation were adjusted by rotations about single bonds until acceptable stereochemistry and packing was achieved. We found that tryptophan B₁₅ can indeed be directed into the relaxin core with no contacts unacceptable in relation to the precision of the insulin coordinates. This and the equivalent insulin structure are shown in Figs 2b and 2a.



Some alternative arrangements of buried side-chains are possible without adjustment of the main chain. An advantage of the computer graphics system is that such alternatives can be rapidly explored. In particular, we found that tryptophan B₁₅ can be orientated in a second distinct way (Fig. 2c) related principally by 180° rotation about the C_β-C_γ bond, and corresponding essentially to that reported by Bedarker *et al.*⁴. It gives a rather more open hydrophobic core than our preferred alternative of Fig. 2b.

We built the terminal stretches of chain beyond the disulphide bridges as they occur in insulin. The additional residues at the N termini were built in arbitrary but sensible conformations giving reasonable contacts to the main body of the molecule. There were two difficulties at the B-chain C-terminus. The sequence reported by Schwabe *et al.*² differs from that of

Fig. 1 a, The amino acid sequences of insulin and relaxin; **b**, the insulin main chain viewed from a direction that illustrates the relative positioning of the central B-chain α helix and the two roughly antiparallel A-chain helical stretches. The hydrogen bonds in the well defined B-chain α helix and subsequent turn are dotted; we expect this structural element to be least affected by the sequence changes in relaxin. *, Residues which seem to be crucial to the integrity of the insulin structure and which differ in relaxin. The extensions at the N termini in relaxin relative to insulin are represented as ribbons, the deletions at the C termini by dashed lines.

James *et al.*¹ from B₂₁ onwards, possibly because of microheterogeneity. (This does not affect the central region of the molecule.) We have followed the sequence of James *et al.* and for simplicity identify relaxin residues by their position in insulin. In insulin, B₂₃ is glycine and its substitution by an L-amino acid (serine in the sequences of James *et al.*, valine in that of Schwabe *et al.*) would give impossible contacts, so there must be a conformational difference here between relaxin and insulin. We continued beyond B₂₃ in a way which left the residues near the positions of their equivalents in insulin. In contrast to B₂₃, the glycines at B₈ and B₂₀ are preserved in relaxin.

The main features of our proposed model are: (1) of the two tryptophans, B₁₅ is buried while B₂₅ is exposed. This fits the chemical and fluorescence observations of Schwabe and Braddon⁹, who found that one tryptophan reacted readily without altering the hormone's potency, while reaction of the second was very difficult and inactivated the hormone; (2) there are compensating changes in side chains that are spatially related, such as B₆ and B₁₄ and A₂ and A₁₆, which are consistent with insulin and relaxin having the same internal organisation¹¹ (see Fig. 1a); (3) there is complementary distribution of the many charged side chains, particularly noticeable on the helical stretches. This not only supports the proposed folding but also suggests that the relaxin molecule achieves stability as a monomer through these favourable interactions¹⁰; (4) detailed changes near B₂₃ modify the characteristics of the monomer surface involved in dimer formation in insulin; (5) residues B₉ to B₁₉ fit well in an unbroken α helix, so that the

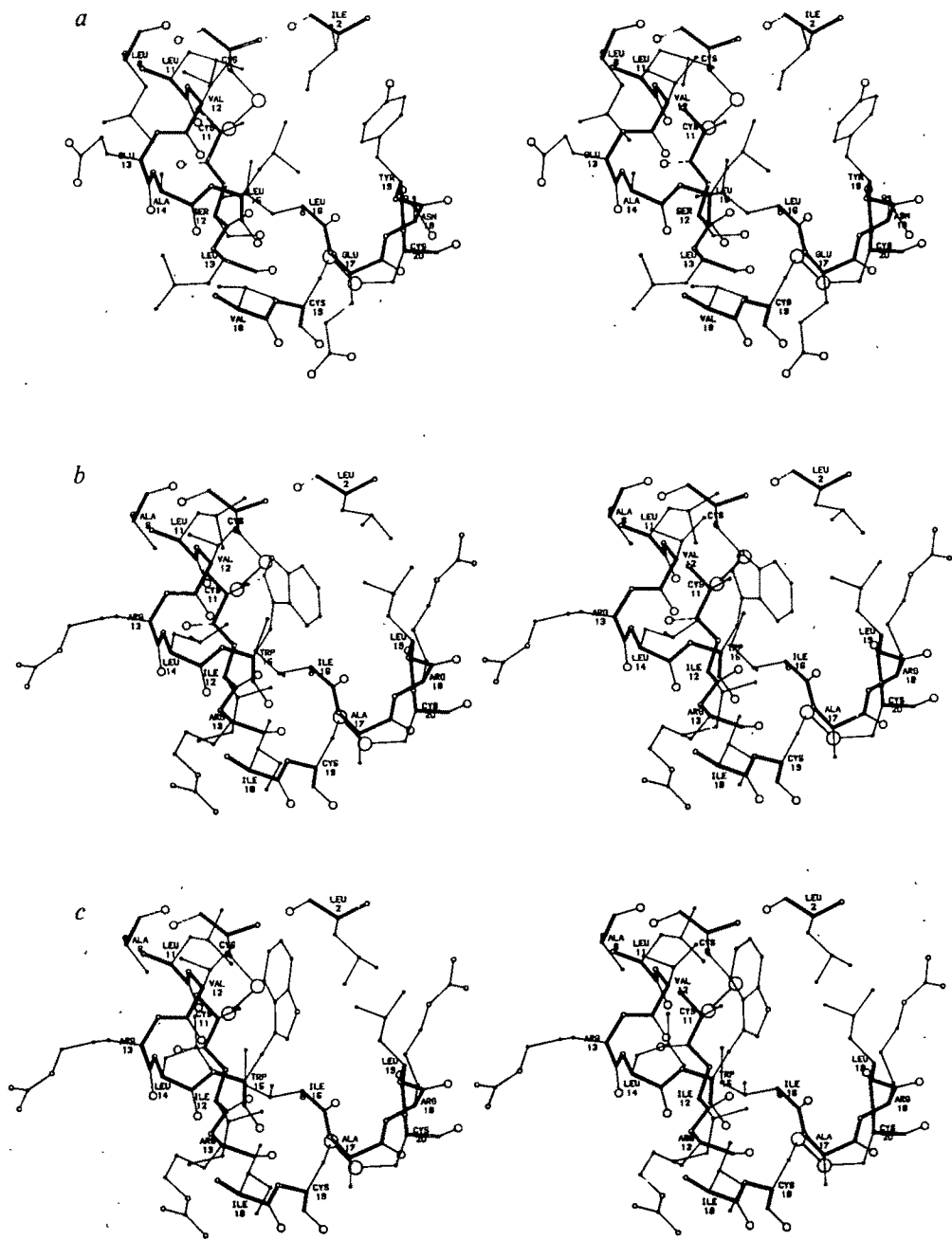


Fig. 2 Buried residues in the central core: *a*, 2-zinc insulin; *b* and *c*, alternative arrangements of Trp B₁₅ in relaxin. The A chain is shown by open bonds and the B-chain by solid bonds.

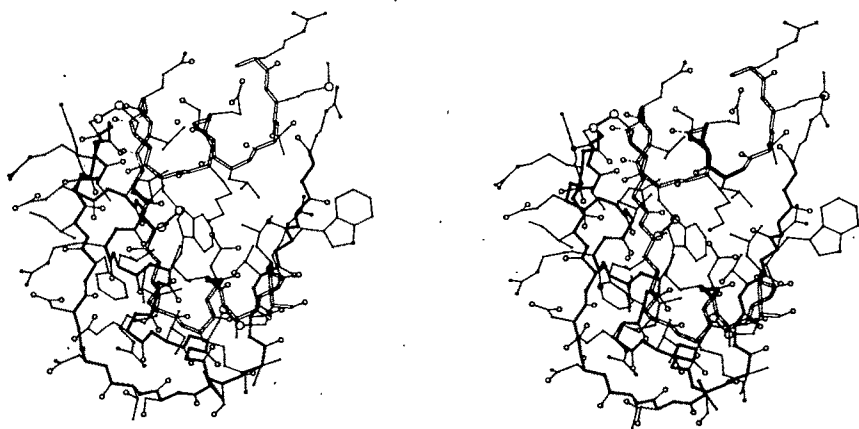


Fig. 3 A stereo view of the complete relaxin molecule with B₁₅ as in Fig. 2*b*.

improvement in sequence homology achieved by displacing the two sequences at B₁₄ to B₁₇ (ref. 1) seems to have no significance in structural terms.

We consider that relaxin has a three-dimensional organisation similar to that of insulin. We do not yet know the closeness of this relationship but note that insulin itself shows considerable structural variation⁶. Thus, while the B-chain α helix is closely preserved, we find widely different conformations of the terminal residues and some alterations of the A-chain helical structure. Therefore, we might expect greatest similarity between relaxin and insulin in the α -helical B-chain and increasing differences in the A chain and the chain termini.

Amongst the many sequence differences between insulin and relaxin, there are two of particular relevance to function. The first is the absence of residue A₂₁ in relaxin. When A₂₁ asparagine is removed from insulin so that, like relaxin, its A-chain terminates at A₂₀ cystine, the hormone's solution and spectral properties are profoundly altered¹¹, its A-chain is apparently perturbed¹² and its biological potency is much reduced^{11,14}. Second, the substitution of B₂₃ glycine by an L-amino acid has been shown by the Chinese studies effectively to abolish insulin's biological activity¹⁵. In diverging from insulin at A₂₁ and B₂₃, relaxin therefore will be without the capacity to behave like insulin. (We note that in the insulin-like peptide responsible for the non-suppressible insulin-like activity in the serum (insulin-like growth factor), B₂₃ is glycine and A₂₁ is alanine, though this is not the C-terminal amino acid¹⁶).

X-ray analysis of relaxin and other hormones with recognised homology to insulin¹⁷ could clarify the relationships between their sequences, structures and biological behaviour.

We thank Professor T. L. Blundell for showing us the paper by Bedarkar *et al.* before publication. The coordinates of our model have been deposited with the Protein Data Bank.

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Received 19 September; accepted 23 November 1977.

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A novel continuous sequence of 41 aspartic and glutamic residues in a non-histone chromosomal protein

THE structural functions of four of the five histones are now partially understood¹ and the nucleosome structure of chromatin well established^{2–4}. However, superimposed on this basic nucleosome structure is some heterogeneity with respect to the other chromosomal proteins, since although it has been shown that isolated nucleosomes contain non-histone chromosomal proteins^{5,6}, there are insufficient of some classes for one per nucleosome. These nucleosomal non-histone chromosomal proteins have been designated the high-mobility group (HMG) proteins. They have been fractionated, and the main components, HMG 1, HMG 2, HMG 14 and HMG 17 have been isolated in a pure form^{7–9}. The complete amino acid sequence of one of these proteins, HMG 17, has been determined¹⁰. We report here that the C-terminal region of protein HMG 1 contains an extremely unusual sequence of amino acids—41 aspartic and glutamic acid residues.

The molecular weight of HMG 1 as determined by sedimentation equilibrium, is approximately 26,000. One of the interesting features of HMG 1 is the fact that over 50% of its amino acid residues are charged. Like the histones, 25% of the residues are basic. However, unlike the histones this protein also contains 30% acidic amino acids. Because of the quantities present in the nucleus (approximately 10⁶ molecules), we feel that, like the histones, HMG 1 is a structural protein and is probably not involved in specific gene control.

Previous work has shown that the acidic amino acids in HMG 1 are irregularly distributed within the molecule¹¹. A cyanogen bromide peptide, CB2, approximately 120 amino acid residues in length, has been isolated from HMG 1 (ref. 11). This peptide contains 47% aspartic and glutamic residues, and because of the absence of homoserine or homoserine lactone presumably comes from the C terminal of HMG 1. The sequence of the first 44 residues of this peptide has been determined on a protein sequenator (unpublished results). As this sequence contains only 10 aspartic and glutamic residues (23%), the majority of the aspartic and glutamic residues must, therefore, be concentrated in the C-terminal portion of this peptide. We present here evidence that the aspartic and glutamic residues in the C-terminal region of this peptide are, in fact, present as a region of 41 aspartic and glutamic acid residues in continuous sequence.

The elution profile of a tryptic digest of peptide CB2 on a Sephadex G-25 column is shown in Fig. 1. The elution profile shows a peak (T) which elutes just within the excluded volume of the column. This peak was further purified by descending chromatography on Whatman 3MM paper in butanol–pyridine–acetic acid–water (90 : 60 : 18 : 72) for 7 d. A single peptide (T1) moved about 1 cm from the origin leaving a small amount of ninhydrin-positive material at the origin. This peptide (T1) was eluted with 5% acetic acid and lyophilised. The amino acid composition of peptide T1 was determined as Lys₃, Asx₁₂, Glx₂₆ and the N-terminal amino acid shown to be lysine by dansylation. The amino terminal sequence of this peptide was determined on a protein sequenator and was shown to be Lys-Lys-Glu-Glu-Glu-Glu-Asp-Glu-Glu-Asp-Asp-Glu. As this peptide was produced by tryptic cleavage of CB2, a lysine residue would be expected at the C terminal of this pep-

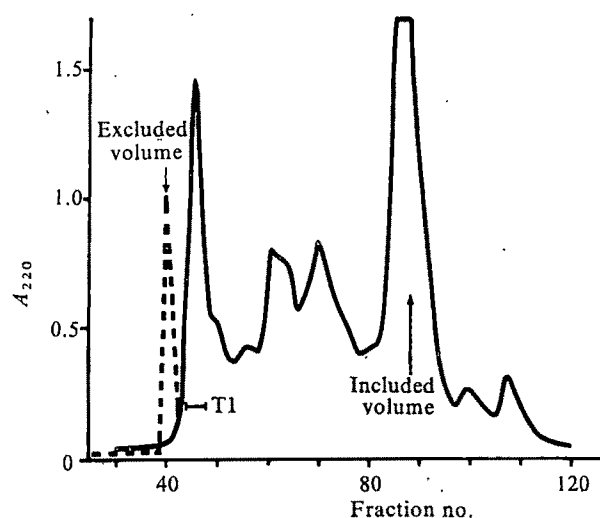


Fig. 1 Sephadex G-25 (90 × 2.4 cm) elution profile of the tryptic digest of CB2. For the digest, peptide CB2 was dissolved in 0.2 M ammonium bicarbonate (10 mg ml⁻¹) and incubated at 37 °C at an enzyme to peptide ratio of 1 : 50 (w/w) for 4 h. The digest was lyophilised and run in the column in 0.01 M HCl: 5 ml fractions were collected.

tide. This peptide could not end at the C terminal of HMG 1 as we have previously shown the C-terminal sequence of HMG 1 to be Phe-Ala-Lys¹². It therefore seems, from the amino acid composition of peptide T1, that we have a continuous sequence of 41 aspartic and glutamic residues with a lysine residue at each end. This peptide was taken through 25 cycles on the sequenator, and although unambiguous identification of the sequence beyond step 12 was not possible, no asparagine or glutamine residues were observed on any of the cycles. Additionally, it is unlikely that any of the aspartic or glutamic residues in this peptide are amidated, since we have previously shown¹² that only about 11 aspartic and glutamic residues are amidated in the total molecule, and we have found this number of amidated residues in other peptides during our sequence studies on this protein. The known difficulty of removing the thiazolinones of aspartic and glutamic acid from the sequenator cup at the butyl chloride wash step presumably contributed to the considerable amount of overlap that was evident by cycle 12. Both aspartic and glutamic acid residues were identified on the later cycles, but it was not possible to determine an unambiguous sequence.

We have previously shown that peptide CB2 does not bind to DNA¹¹, unlike total HMG 1. This is not surprising considering the acidic nature of this peptide. It seems likely, however, that this region will combine with basic parts of other chromosomal proteins and may even compete with the DNA and possibly break down the nucleosome structure. Interference with the DNA-histone interactions, or possibly some form of higher order structure, or cross-linking, would seem the most likely functions for this molecule, considering that there is only sufficient HMG 1 for approximately one in six nucleosomes⁵.

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Received 23 September; accepted 23 November 1977.

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Electron density map of apoferritin at 2.8-Å resolution

THE structure of ferritin is of considerable interest because of its widespread occurrence in higher organisms, signifying a general need to store iron and remove this essential, but toxic element. Horse spleen apoferritin has a molecular weight (MW) of about 444,000 and is composed of 24 subunits (MW 18,500) each containing about 163 amino acids¹. These are arranged in 432 symmetry to form a nearly spherical hollow shell with outside and inside diameters approximately 130 Å and 75 Å respectively¹⁻³. The large cavity inside the molecule can store up to 4,500 Fe atoms, packaged in a microcrystalline inorganic component of approximate composition (FeOOH)₈(FeO:OPO₃H₂) (refs 4-6). The atomic structure of the micro-crystals is not specifically related to the surrounding protein structure⁶. Apoferritin catalyses the oxidation of Fe(II) which it retains inside the molecule as the ferric hydrolysate^{7,8}. Our 6-Å resolution structure of apoferritin⁹ showed the presence of several rods of electron density, tentatively assigned as α helices, and channels passing through the shell, which could provide an access route for Fe atoms. These features have been confirmed at 2.8-Å resolution and we can now also provide a plausible subunit conformation and quaternary structure.

Ferritin was purified from horse spleen and reduced with sodium dithionite at pH 4.8 (ref. 2). Apoferritin crystals (F432, *a* = 184 Å, *Z* = 4) were grown as previously described³. Two heavy atom derivatives used for phase determination were prepared by soaking crystals in 1% CdSO₄ solution containing either *p*-chloromercuribenzoate (PCMB) or K₃UO₂F₅.

Data to 2.8-Å resolution were collected on an Arndt-Wonacott oscillation camera using monochromatised Cu Kα radiation. Heavy atom parameters were refined using full matrix least squares refinement on centric data. Phases for all reflections were calculated by the method of Blow and Crick⁹ using anomalous scattering information¹⁰. The mean figure of merit for the phase set was 0.62. Details of heavy atoms derivatives used are shown in Table 1.

The electron density map, small portions of which are shown in Fig. 1, shows that the subunits are arranged within the

Table 1 Principal heavy atom parameters after refinement

Derivative	OCC†	<i>x/a</i>	<i>y/b</i>	<i>z/c</i>	<i>U</i> _{iso} (Å) ²
PCMB	0.488	0.1610	0.1783	0.1999	0.503
(<i>R</i> _c = 55%)	0.271	0.1216	0.2130	0.0240	0.584
	0.089	0.0754	0.1234	0.2720	0.1‡
K ₃ UO ₂ F ₅	0.164	0.0154	0.1489	0.1738	0.433
	0.093	0.0640	0.0766	0.2156	1.086
(<i>R</i> _c = 62%)	0.071	0.1136	0.1743	0.1692	1.49
	0.060	0.0150	0.1375	0.1875	0.5‡
	0.080	0.0500	0.0930	0.2190	0.5‡

*Crystallographic residual $R_c = \frac{\sum (|F_{PH} - F_P| - |F_H|)}{\sum |F_{PH} - F_P|}$

†Relative occupancy, at an arbitrary overall scale factor.

‡Parameter not refined.

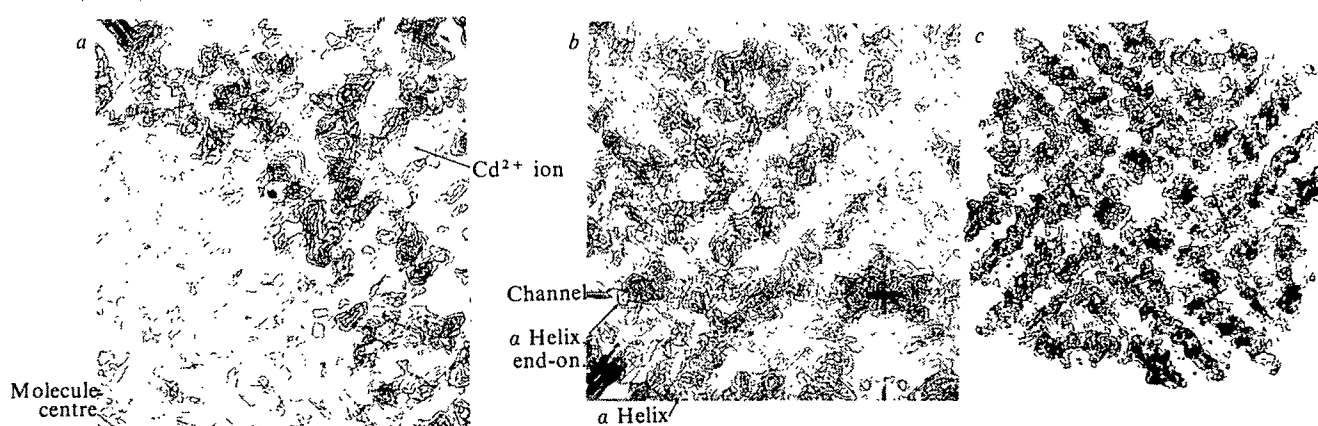


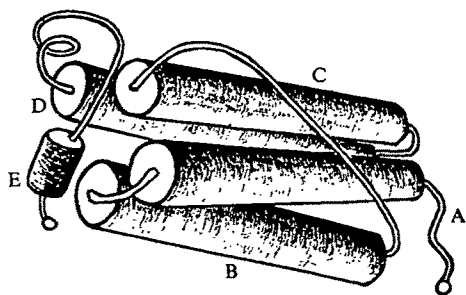
Fig. 1 Sections of the 2.8-Å resolution electron density map of horse spleen apoferritin viewed nearly parallel to the fourfold axis. Each part of the map shown comprises sections at intervals of $1/132$ of the unit cell edge, with contours at equal and arbitrary levels. A complete slice through the molecule is obtained by operation of the fourfold symmetry axis. *a*, Eight sections near the centre of the molecule. The molecule is seen as a hollow shell, two layers of helices thick. Two peaks related by a unit cell dyad (dotted line) form a double bridge between neighbouring molecules in the crystal. We attribute these to Cd^{2+} of crystallisation. The full circle near to the twofold axis, but on the inner surface of the shell shows the position found for a Tb^{3+} ion in a separate isomorphous replacement study. *b*, Nine sections near the top of the molecule. The left hand edge of the map cuts through one of the six channels which penetrate the shell along the fourfold axis. The subunits of the shell each comprise four long α helices (some shown in the illustration) and a short helix. The short helices from adjacent subunits surround the channels and can be seen on end. *c*, A complete slice about 100 Å across and 10 Å thick through the apoferritin electron density map near the top of the molecule viewed down a fourfold axis. Scale of (*a*) and (*b*) approximately $1.6 \times$ that of (*c*).

protein shell so as to form a bilayer of α helices enclosing the central cavity (the iron core in ferritin). The helical axes lie perpendicular to the radius vector, hence one layer of helices faces inwards and the other outwards.

Subunits are roughly cylindrical with h about 55 Å and d about 27 Å. The main structural features, accounting for about two-thirds of the primary structure, are four very long α helices (34–42 Å) with their axes nearly parallel, making up most of the cylinder length. There is an additional short length of helix perpendicular to the major helices. We have attempted to trace the complete course of the polypeptide chain in the electron density map and to determine its direction, but in the absence of sequence information, this cannot be done with complete confidence. However, although there are some ambiguities in inter-helical connectivities, which lie at the molecular surfaces, a plausible interpretation is shown in Fig. 2. In this structure the four long helices form two anti-parallel pairs. Helices B and C seem to be connected at their opposite ends by a long, extended loop, such that the pairs of helices are related by a pseudo-dyad perpendicular to their lengths. The short helix, E, probably lies at one of the chain termini.

We have tentatively assigned the N-terminus to the end of a

Fig. 2 Schematic drawing of an apoferritin subunit. The subunit contains four nearly parallel helices 34–42 Å long and a short helix, E, at right angles to these. Four of the short helices, E, line the channels while the long helices lie along the molecular surfaces. Non-helical segments connecting the helices are indicated. These have been derived from the map and provide a plausible subunit construction, although the connectivities cannot as yet be assigned with certainty.



short non-helical segment attached to helix A. The long connecting loop between helices B and C lies over the surface of the apoferritin molecule. Proteolytic attack at this accessible loop could account for the 6,000–8,000 and 10,000–12,000 MW fragments sometimes observed on polyacrylamide gel electrophoresis in the presence of sodium dodecyl sulphate^{11,12}. The tertiary structure of a subunit superficially resembles myohaemerythrin¹³, which also contains two anti-parallel pairs of helices, and the tobacco mosaic virus (TMV) subunit¹⁴, but in TMV subunits pack with their helices lying radially rather than tangentially as in apoferritin.

The packing of subunits into the quaternary structure of apoferritin suggests the formation of stable dimers as intermediates in self-assembly. Subunits related by molecular dyads overlap along most of their length (Fig. 3*a*) with a larger mutual area of contact than those around threefold (Fig. 3*b*) or fourfold axes (Fig. 4*b*). Eight helices in the dimer lie roughly parallel to one another. Contacts between subunits around fourfold axes are relatively tenuous and stable symmetrical tetramers would not be expected (Fig. 4*b*). Operation of the fourfold axis produces approximately 10 Å-wide channels through the protein shell noted at low resolution³ (Fig. 4*a, b*), which are lined by short helices (E in Fig. 2), seen end-on in Fig. 1*b* and *c*.

Around the threefold axis the contact region is greater than at the fourfold. The cylinders form a three-blade propeller arrangement, with one end of the cylinder lying approximately perpendicular to the cylindrical surface of its symmetry-related neighbour (Fig. 3*b*). Formation of hexamers by aggregation of dimers around the trigonal axis might be expected to be a second stage in assembly. The subunit interactions are reminiscent of those in methaemerythrin, where parallel and perpendicular cylinder stacking is also found¹⁶, although the overall symmetry and number of inter-subunit contacts differ. A schematic drawing of the apoferritin quaternary structure viewed down a fourfold axis is shown in Fig. 4*a* in which it can be seen that each subunit is in contact with five neighbours.

Several metal binding sites can be located with respect to the subunit structure. The major PCMB site is near the threefold axis, towards the outside of the molecule. Other sites are near either the molecular or cavity surfaces. No metal ions have been found embedded within the shell either within or between subunits. Pairs of peaks, which we attribute to Cd^{2+} of crystallisation, occur close to the twofold axes on the outside of the molecule and connect adjacent molecules in the crystal

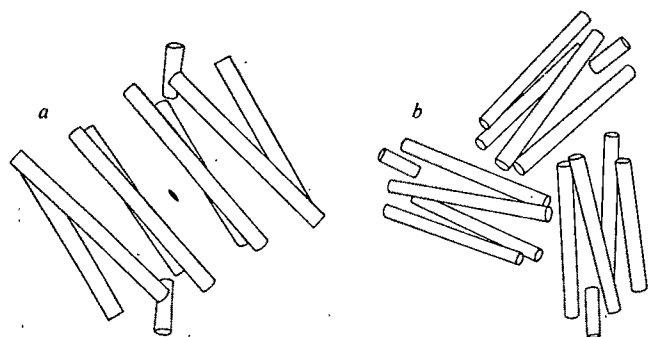
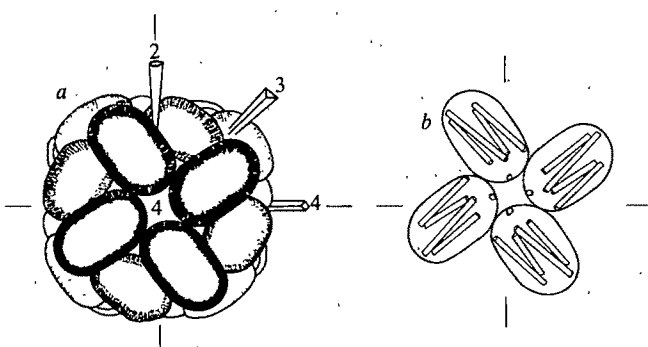


Fig. 3 Schematic views of apoferritin subunits, showing local packing around *a*, molecular twofold axes, *b*, molecular threefold axes. Only helical regions are shown. The helical rods from neighbouring subunits related by molecular dyads overlap along most of their lengths, and give a system of eight nearly parallel rods. A symmetrical dimer is thus suggested as a stable intermediate in the assembly of the protein shell. Probable inter-helical connections suggest that chain directions alternate so that neighbouring helices are antiparallel. Subunits related by molecular threefold axes have their helices nearly perpendicular and there is less inter-subunit contact than around the twofold axes.

(Fig. 1*a*). The Cd^{2+} ion pairs are bound roughly half way along the extended BC loop. Four loops and two Cd^{2+} ions participate in the double intermolecular bridge.

Not used in the structure determination, but found in separate isomorphous replacement studies are Tb^{3+} ions on the inner surface of the shell. The major site (Fig. 1*a*) is close to the twofold axis at a radius of 41.6 Å and only 4.3 Å from its symmetry-related pair. The Tb^{3+} sites which probably involve carboxyl groups¹⁵, are of special relevance to ferritin formation. Our mechanism for the catalytic action of apoferritin in the oxidation of Fe(II) , which gives it the power to accumulate Fe(III) , becoming ferritin, involves sites for Fe(II) -oxidation and heteronucleation of hydrous ferric oxide⁸. Tb^{3+} ions inhibit iron uptake by apoferritin, and may bind at Fe-binding sites.

Fig. 4 Schematic drawings of the subunit arrangement in an apoferritin molecule viewed down a fourfold axis. *a*, Complete molecule showing some of its symmetry axes. *b*, Subunits related by a fourfold axis. Subunits approximate to cylinders or ellipsoids with length (approximately 55 Å) about twice their diameter (approximately 27 Å), although the shell is rather more smooth than these shapes suggest. Each subunit is in contact with five neighbours, although the contacts are of only three different types around the three different symmetry axes (as in Figs 4*b* and 3*a*, and 3*b*). The four major helices present in each subunit have their axes roughly parallel to the long axes of the subunits as drawn schematically in *b*. The interactions between subunits making the symmetrical tetramer seen in *b*, are less extensive than those in the symmetrical dimer and trimer of Fig. 3*a* and *b*. Compare with Fig. 1*c*.



The presence of a double site suggests the possibility of a cooperative two-electron transfer to oxygen from two close Fe(II) , giving two Fe(III) , which is followed by the formation of an oxo-bridge by elimination of protons from bound water molecules. Here again we can compare the structure with that of myohaemerythrin¹³, in which each subunit contains a pair of Fe atoms 3.44 ± 0.05 Å apart, which reversibly binds oxygen. This Fe-Fe pair is set well within the subunit, whereas in apoferritin the Tb^{3+} ions belong to different subunits and lie on the inner surface of the protein shell.

We thank the SRC, MRC and the Royal Society for financial support. We thank Drs H. Watson and H. Muirhead and their colleagues at Bristol for assistance and for providing facilities for X-ray film measurement and processing.

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Erratum

In the letter 'Subgenomic, cellular Rous sarcoma virus RNAs contain oligonucleotides from the 3' half and the 5' terminus of virion RNA' by P. Mellon & P. H. Duesberg, *Nature* 270, 631, lines 32-35 of paragraph 4 should be deleted. In the last line of paragraph 4 the numbers 6 and 3 should be underlined.

Corrigendum

In the letter 'On melting icebergs' by H. E. Huppert & J. S. Turner, *Nature* 271, 46, in the first line for 'their' read 'suitable'.

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reviews

Pulsar compilation

F. Graham Smith

Pulsars. By R. N. Manchester and J. H. Taylor. Pp. 281. (Freeman: San Francisco and Reading, 1977.) \$9.95.

THE ten years since the discovery of the pulsars have seen the publication of a bewildering flood of information on their properties, a plethora of theories about how they work, and a rather more coherent set of papers on their distances, their population within the Galaxy, and on the effects of the interstellar medium on the propagation of their radio pulses. Drs Manchester and Taylor have between them contributed a large proportion of the observational material now available to us, and it is very helpful to find so much assembled in one volume.

The difficulty with the pulsars is that we do not really know how they pulse; more precisely, we do not know how they produce the searchlight beam which crosses our line of sight each time the underlying neutron star rotates. The book cannot therefore start with an exposition of the structure of a pulsar, working from the core of the neutron star outwards, because it would be in difficulty as soon as it left the surface and entered the magnetosphere which is the seat of the observed phenomena. The authors therefore take the course of piling the observations into the first two-thirds of the book, leaving the reader dazzled with a rich assortment of phenomena, and presenting the theories in the remaining third. The theories have turned out to have almost the same richness and variety as the observations themselves. Here is the problem: should the authors select what they regard as the key observations, indicate those theories that can possibly accommodate them, and throw out the theories that now seem impossible? The alternative, which the authors adopt, is to keep an open mind and trust that the reader will be able to do the sorting out for himself. As a consequence, the origin of the pulses moves, from page to page, and even within a page, from near the surface to outside the velocity of light cylinder without much guidance to the reader.

The chapters on the interstellar medium and the population of pulsars are very useful and clear expositions. There are good discussions of the Crab

Nebula, and on the relativistic effects to be expected in observations of the binary pulsar. The chapter on X-ray pulsars is a useful introduction, although the subject grows so rapidly that it is already out of date.

The observational material includes some previously unpublished work, and is up-to-date in including the optical observations of the Vela Pulsar, made in January 1977. This latter work, incidentally, has been inserted into a

chapter on X-ray pulsars and binary systems, neither of which is an appropriate category.

Given that there is as yet no key to unlock the main mystery of the pulsars, this book is welcome as a compilation and an authoritative commentary. There is a good index to help one find one's way around. □

F. Graham Smith is Director of the Royal Greenwich Observatory, Herstmonceux Castle, Sussex, UK.

Protein chemistry for immunologists

Immunochemistry of Proteins. Vols 1 and 2. Edited by M. Z. Atassi. Pp. 438 and 485. (Plenum: New York and London, 1977.) \$53.40 each volume.

THE first volume is a hotch-potch of a book, masquerading under a false title and falling between a definitive work and a collection of contributors' 'pet topics'. Over half of the book is devoted to chapters by the editor himself (M. Z. Atassi) and a colleague (A. F. S. A. Habeeb), on chemical modification and conformational aspects of proteins, respectively. And much of what they say could hardly be considered as immunochemistry according to the accepted meaning of the term. Habeeb does, however, make a few comparisons between the structural and antigenic relationships of proteins of different species of origin, besides offering some reflections about the application of immunochemistry which include the somewhat obvious conclusion that "... advances in immunochemical research in the last two decades resulted from the development of new techniques and methodologies".

The selection of the topics treated by most of the other authors has obviously been on an arbitrary basis. For instance, along with useful contributions on histocompatibility antigens (still very much in the throes of characterisation) and the antigenicity of collagen and encephalitogenic proteins, there is but one chapter dealing with the study of antigen-antibody interaction (by the somewhat specialised fluorescence polarisation technique). There is also a chapter on lymphoid cell responses to protein and peptides. All in all then a 'very mixed

bag' of subjects, of variable immunochemical appeal.

The recent appearance of a second volume in the series has done little to mollify these types of criticism. Again, there are useful practically orientated chapters, such as the one by Parikh and Cuatrecasas on affinity chromatography and other more theoretically inclined ones dealing with topics like "the effect of antigen structure on immunogenicity". There is also a chapter on Concanavalin A.

Over half of this volume, however, is devoted to contributions on the antigenic structures of those proteins which have now been well characterised, that is, myoglobin, lysozyme and tobacco mosaic virus. And, although the major contributions to this field by Atassi and his coworkers serve as valuable models to those engaged in the similar characterisation of other proteins, one cannot help gaining the impression that these two volumes are mainly outlets for wider propagation of such approaches to structural characterisation rather than attempts to provide well balanced texts on immunochemistry. Where, for example, is any treatment of the contribution of the antibody to immunochemical reactions, or of other ancillary proteins like the complement components?

Perhaps such topics will be considered in later volumes. In the meantime, a fairer title of the present two volumes would be *Protein Chemistry for Immunologists*, but even then the coverage is 'uneven' to say the least.

D. R. Stanworth

D. R. Stanworth is Reader in the Department of Experimental Pathology at the University of Birmingham, UK.

Mathematical conversion kit

Mathematics Applied to Continuum Mechanics. By Lee A. Segel. Pp.xviii +590. (Macmillan: London and New York, 1977.) £14.25.

ALTHOUGH the general tradition of mathematics departments in the US had been rather specialised towards pure mathematics, the best American universities have recognised for many years now the need for Applied Mathematics Programs. These have been aimed, especially, at helping those who, although brought up in the pure mathematics tradition, would like to consider the possibility of transferring their interest to fruitful areas of application of mathematics.

For example, one of the finest and oldest of the world's technological universities, Rensselaer Polytechnic Institute in Troy, New York, has given an introductory applied mathematics course to its mathematics students for ticity theory contributed by G. H. Handelman initiated it. It was later developed by Dr L. A. Segel, whose book *Mathematics Applied to Continuum Mechanics* in 12 chapters (with the material of chapters 4-6 on elasticity theory contributed by G. H. Handelman) has grown out of the course.

Applications of mathematics fall into two main areas. One involves the discernment and utilisation of laws of nature, as illustrated by Dr Segel's book or by its more elementary predecessor *Mathematics Applied to Deterministic Problems in the Natural Sciences* (C. C. Lin and L. A. Segel (Macmillan: London) A contrasting area of application is to statistical treatments of observed regularities and variabilities in systems where no underlying laws are known. Both are of immense practical utility, and it is important that mathematical education should make students aware of both.

In Britain, those taking mathematics in the immediate pre-university years have commonly had that advantage, being exposed both to mechanics and statistics as well as to pure mathematics, and their university course has built on that background and developed considerably within the area of mathematics applied to engineering and the natural sciences, as well as within statistics and operational research. More recently, in gross neglect of the country's needs, many schoolteachers have taken away from students the uniquely valuable example of elementary mechanics as an area illustrating mathematics applied to problems in engineering and the natural sciences, where natural laws are discerned and

utilised. Consequently, students enter university mathematics departments without any knowledge of this great division of applied mathematics, and with an educational background based on pure mathematics with some leavening of statistical distribution theory.

For the first time, then, British universities may be facing the problem which has long existed in the US, and which the best American universities have been seeking to counter with books like those of Dr Segel. Such books, directed rather explicitly at students with a good modern background in pure mathematics, but with little or no idea of classical applied mathematics or the uses of mathematics in engineering science, are aimed at capturing the interest of such students, and at bringing them to begin thinking in ways valuable to such applications.

Exercises abound throughout this excellent book and are effective (with or without use of the 'hints' section at the end) as a major method of exposition of material. Part A gives a good grounding in vector and tensor algebra and calculus, with particularly clear explanations of why all the definitions are appropriate. Part B gives a good introduction to rate-of-strain, viscosity, vorticity and boundary layers; and to strain, elastic constants, energy and uniqueness principles, bending, buckling, torsion, plane strain, generalised plane stress, and elastic waves.

Physical variability of the oceans

Variability of the Oceans. By A. S. Monin, V. M. Kamenkovich and V. G. Kort. Pp.xiii + 241. (Wiley-Interscience: New York and London, 1977.) £14.95; \$25.35.

THIS monograph by three well-known Soviet oceanographers is a survey of the physical variability of the oceans on widely varying space and timescales. Although the writing is clear and the errors few, I suspect that the book will have a limited readership.

The major shortcoming is that the most recent reference to the Western literature is 1973, and only 6% of all Western references are dated after 1970. Soviet citation runs a bit later, as one would expect. As any casual observer of physical oceanography will have noticed, ocean variability has probably been the area of greatest advance since 1970, and understanding of many parts of the spectrum has improved markedly since then. Furthermore, oceanography is a very technologically dependent subject, and it is in technology that the Soviet Union most obviously lags

Part C, on water waves, is concerned mainly with the deep-water case. There is a good introduction to group velocity. For example, the crest pattern is found not only for gravity waves generated by a large obstacle in steady motion (ship waves) but also for the limiting case of a very small obstacle making capillary waves. The author calls these 'beetle waves' in reference to a pretty frontispiece photograph! Part C concludes with a brief but interesting treatment of second-order effects. For me, only its opening chapter, deriving the free-surface condition and other basic equations, seemed to me to adopt an unnecessarily cumbersome approach based on excessive adherence to 'scaling' doctrines.

Finally, Part D is concerned with setting out the calculus of variations and its important applications in mechanics, and continues the excellent practice of getting the reader to work out the details in most of the really interesting examples. Wherever universities are faced with 'converting' students grounded primarily in pure mathematics to the great variety of its exciting and important applications in the mechanics of solids and the mechanics of fluids, I believe that they will find this book a most valuable conversion kit.

James Lighthill

Sir James Lighthill is Lucasian Professor of Applied Mathematics at the University of Cambridge, UK.

behind the West. Thus the more recent Soviet references do not make up for the dated discussion of Western work to the extent that one might otherwise have expected. The commonly remarked Soviet strength in theory does not compensate in this field for their missing observational strength, but does lend a formal tone to much of the book.

A good textbook need not be fully modern, and oceanography painfully lacks such textbooks. Students trying to enter oceanography, however, will find the subject matter here too terse. For those already conversant with the field, the obsolescence will be a serious defect. Perhaps the book is best used as an annotated bibliography of material through 1970. It also has historical value, as it provides insight into the thinking of some of the most prominent Soviet oceanographers in the early 1970s. In many ways, the book reads like the type of overall summary of a field that has become a commonplace in recent years as a preamble to and justification for large cooperative national and international field programmes. I suspect that is its origin.

Carl Wunsch

Carl Wunsch is Professor of Physical Oceanography and acting Head of the Department of Earth and Planetary Sciences, Massachusetts Institute of Technology.

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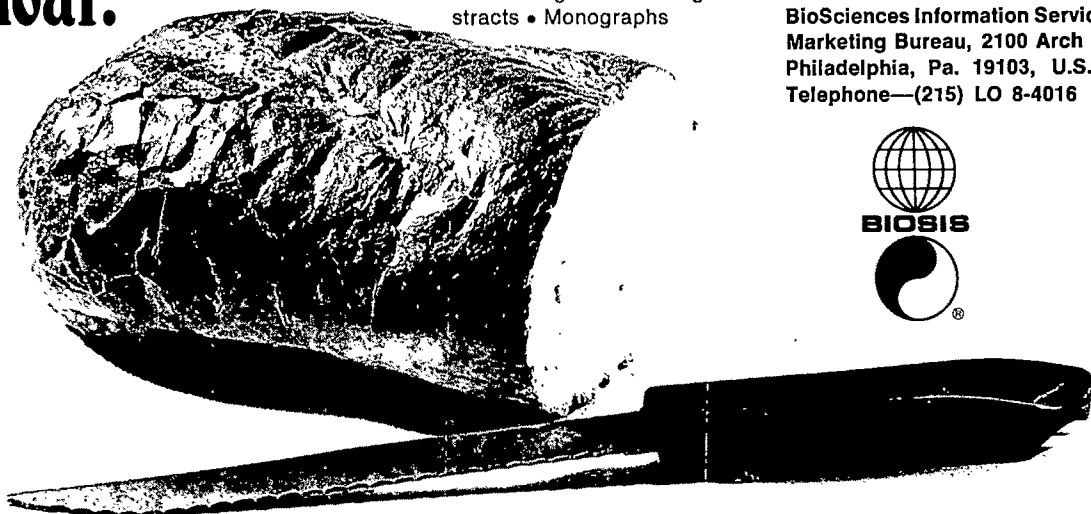
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Physical aspects of biological systems

Biophysik: Ein Lehrbuch. Edited by W. Hoppe, W. Lohmann, H. Markl and H. Ziegler. Pp. 720. (Springer: Berlin, Heidelberg and New York, 1977.) DM98; \$43.20.

A TEXTBOOK which describes biophysical approaches and physical aspects of biological systems would fill a gap, because most existing books deal with methods preferentially. The present book written by 54 authors, mostly from Germany, takes an interesting step in this direction.

The strongest part of the book is contained in the many chapters on biophysical aspects of various systems written by many well known workers in the field. These contributions range from enzymes as catalysts (Huber), membranes (Fisher, Stoeckenius, Sackmann and Frömter) and receptors (Thurm, Kaissling, Menzel, Snyder and Stieve), through photosynthesis (Renger) and muscle-contraction (Mannherz, Holmes) to complex systems such as information transduction in nerve systems (Creutzfeldt), orientation in flight (Reichardt), biomechanics of flight (Nachtigall) and transport of water in plants (Ziegler)—to mention only a few examples. Most of these contributions are very informative reviews of 10–20 pages written for undergraduate students or scientists who wish to broaden the scope of their knowledge. They provide a convenient source of information for many facets of biophysics and cannot be found in such a concise way in other textbooks. Three purely biological or biochemical chapters on the living cell (Schnepf), chemical structure of biological macromolecules (Tschesche) and biological function of nucleic acids (Zillig) are useful, but the information can be found also in other texts.

Chapters dealing with more general aspects of biophysics such as intra- and intermolecular interactions (Hofacker and Ladik), energy transfer (Dörr and Kuhn), cybernetics (Marko) or evolution (Kuhn and Schuster) do not add up to a complete picture. This part of the book naturally suffers more than the other part from the way it was written, namely by many rather specialised authors. For example, the chapter on interactions, written by two theoretical chemists, emphasises the quantum mechanical approach for the theoretical calculation of hydrogen bonds and charge transfer complexes but leaves out hydrophobic interactions and other important contributions.

Chapters on energetics and kinetics contain a basic sketch of general thermodynamics and fundamental kinetics without much emphasis on biological problems. Sections on thermodynamics of macromolecules in solution, multiple equilibria, polyelectrolytes, and so on, would have been valuable. Surprisingly the dynamic aspect of the function of biological macromolecules are not treated, although a good section on fast kinetic methods (Rüppel) is included.

A chapter on physical methods for the investigation of biological macromolecules is of heterogeneous quality. The description of methods for solutions cannot compete with good intro-

ductionary texts such as Van Holde's *Biophysical Chemistry*.

Lecturers and students who are engaged in courses of biophysics would welcome a text on general biophysics. The present book cannot be recommended as a basic text but it may serve as a supplementary book for advanced reading. A difficulty for a worldwide use of the book arises from the fact that it is written in German. This is an anachronism for a book of this type, but perhaps an English translation is planned.

Jürgen Engel

Jürgen Engel is Professor of Biophysical Chemistry at the Biozentrum of the University of Basel, Switzerland.

Indignation against mercury pollution

Mercury Contamination: A Human Tragedy. By P. A. D'Itri and F. M. D'Itri. Pp. xxii+311. (Wiley: New York, London, Sydney and Toronto, 1977.) £13.45; \$22.80.

THE aim of this book is to raise indignation against environmental pollution in general, and pollution by mercury in particular. Beyond the problem of environmental pollution, there is an historical account on the use of mercury. The story of its medicinal use, especially for the treatment of syphilis (as presented by the authors), is a warning to scientists to analyse all information critically and not to substitute possibilities for facts. Unfortunately, the authors do not draw the same conclusion.

There is no doubt about the environmental impact of mercury pollution in the North American lakes and rivers; there is no doubt about the unacceptably high level of methylmercury in fish caught in some of these waters; there is no doubt that methylmercury is a highly toxic accumulative poison, but these facts are not substitutes for the complete lack of controlled morbidity data for the populations of White Dog and Grassy Narrows Reserves. The lack of morbidity or mortality data, to say the least, is surprising. It is alleged that an official admitted "it was a wonder more Ojibways (of the Grassy Narrows) were not dying because some of them had blood mercury levels that exceeded those of fishermen who died at Minamata". It is also surprising that these Indians had higher blood levels than fishermen at Minamata, as, according to the authors, the highest hair level of the members of the Ojibway tribe was 40% lower than the hair level at which Japanese at Minamata began to show

symptoms. Another reason for surprise is that blood mercury concentrations were not estimated in the victims of the Minamata epidemic. In the same chapter there is another misquotation or uncritical quotation which states that 200 g fish consumed for 3 weeks raises the mercury concentration in the blood to 2 p.p.m. A 70-kg man, however, ought to consume 10.7 mg mercury as methylmercury per day for 3 weeks to produce 2 p.p.m. blood concentration—that is, he should eat fish containing an unbelievably high (more than 50 p.p.m.) concentration of mercury.

These and similar overstatements do not help but rather weaken the impact of the book. Mercury, according to the authors, injures at a lower dose and at an earlier time than scientific evidence indicates. As they suspect that mercury had some role in the death of Napoleon, they bring forward his death before the One Hundred Days and Waterloo to the time of his first exile on the Isle of Elba.

These criticism might seem unjustified, as in the preface the authors made it clear that "The book was not written for scientists who conduct research into mercury pollution", probably because compared with the *Toronto Globe and Mail*, and the *Kenora Daily Miner and News*, the scientists are inferior as creators and absorbers of information. First of all, "The scientific method by definition is partial and incomplete" (which is true); secondly, "local citizens instinctively grasp the problem and insist on resolution while scientists are still trying to ensure that their findings are accurate"; and thirdly, even if they are able to grasp the problem "the scientists carefully qualified statements may confuse the public."

Laszlo Magos

Laszlo Magos is a Senior Scientific Officer at the MRC Toxicology Unit, Carshalton, Surrey, UK.

Quantum pharmacology

Quantum Pharmacology. By W. G. Richards. Pp. xiii+213. (Butterworth: Sevenoaks, UK, 1977.) £12.

THE relationship between the chemical structure of small molecules and their biological effects has been studied intensively for over a hundred years. Apart from its intrinsic interest, it is obviously of enormous practical importance in the design of drugs. In recent years, studies of this relationship have been growing steadily in sophistication. The biological effects can now be described in greater detail, often in terms of binding to isolatable receptors and enzymes rather than gross physiological effects. The structural properties of the small molecules, too, are now discussed with greater precision, considering their three-dimensional conformation and electron distribution rather than simply two-dimensional representations of their covalent structure. In the latter area, the past ten years have seen increasing application of molecular orbital methods to molecules of pharmacological interest, and Dr Richards, one of the leading practitioners of this interdisciplinary art, has now written an introduction to the field.

The stated aim of the book is twofold, to introduce medicinal chemists to molecular orbital calculations and to introduce theoreticians to the pharmacological problems to which their methods can be applied. Thus, the book begins with an outline (in 87 pages) of some basic pharmacology, lucidly presented though necessarily very condensed. The emphasis is primarily on the cholinergic and adrenergic systems, histamine and centrally acting drugs, with chemotherapy, prostaglandins and peptide hormones, for example, receiving only brief mention.

The second part (49 pages) introduces molecular orbital calculations. The fundamental properties of wave functions and molecular orbitals are described very clearly, as is their use to calculate molecular properties. There is a valuable chapter in which the various approximate wave functions are described and compared.

In many ways the core of the book is the third section (38 pages), as it is here that the applications of theory to experiment are discussed. The first topic is that of the conformation of the small molecule, illustrated by calculations on a number of neurotransmitters and, in particular, on a series of histamine derivatives. The latter illustrate the "essential conformation approach" developed by the author, in which the biological activity of a series of compounds is correlated with the region of conformational space which they can occupy, and a region of this space which

is in some sense essential for activity is thus defined. Since most conformational calculations consider the small molecule in isolation (*in vacuo*), the next chapter is devoted to a discussion of approaches to the problem of including the effects of solvent (specifically water) in the calculations. The remaining chapters focus on electron distribution—both on empirical correlations with activity, and on the use of the electrostatic potential field of the small molecule as a method of "mapping" the complementary surface of the receptor site (introduced by Weinstein and his colleagues). It is a pity that this section of the book is so brief. It would have been valuable to have a more extensive discussion of some of the many interesting points raised, perhaps at the expense of a shorter pharmacological introduction, for which many introductory texts are available. The book concludes with a very valuable annotated bibliography of all molecular orbital studies of molecules of pharmacological interest reported up to the end of 1976.

Can calculations be useful? The author concludes with a cautious "yes", with which I am sure most readers will agree. The ways in which they are best used remains, to some extent, to be established. There is no question that molecular orbital calculations have revealed interesting correlations between molecular properties and biological activity—as, for example, in the "essential conformation approach" to histamine analogues—and these will continue to be a valuable aid to the rational design of new compounds. What we do not know is how such correlations can be interpreted in physical terms to help build up a picture of the receptor site. There is no doubt that further work in this field will be stimulated and guided by Dr Richards' lucid and critical description of the state of the art.

Gordon C. K. Roberts

Gordon C. K. Roberts is a member of the scientific staff of the Medical Research Council in the Division of Molecular Pharmacology, National Institute for Medical Research, London, UK.

Remote sensing techniques

Aerial Photography and Remote Sensing for Soil Survey. By L. P. White. Pp. viii+104 (Clarendon/Oxford University: Oxford, 1977.) £6.

THE foreword to this book indicates that it is the first of a series of new handbooks following in the footsteps of Robin Clarke's classic *The Study of the Soil in the Field*. This is a disappointing book, mainly because it does not fulfil the expectations aroused by the title and preface. In short, the balance of the book seems to be out of keeping with the topic. Only one chapter (some eleven pages) is directly concerned with soil survey and aerial photography. The remainder of the main text of 93 pages consists of a concise discussion of remote sensing methods, sensors and platforms, which other texts have dealt with in a more comprehensive manner.

Readers concerned with the use of remote sensing techniques in soil survey will turn their attention to chapter 4. Those familiar with image interpretation will doubtless recognise the wide range of issues touched on here. The brevity of the treatment, however, results in a failure to discuss in any depth the many aspects of the relationships between soil mapping units and image characteristics. For example, the treatment of terrain analysis is rather perfunctory, and the topics of pedo-

logical analysis and photo-interpretation keys merit further discussion. A much fuller treatment of the value of images/photographs for the detection of temporal variations in soil conditions (for example, structure and profile drainage) would have been useful. Similarly, more attention could have been given to definitions of soil mapping units, their relationships to different scales of imagery, and the testing of the accuracy of interpretation.

The bulk of the book comprises descriptions of photography and photographic products, line-scanners, side looking radar, imagery from space platforms, image enhancement, and automatic analysis. There is, however, little significant comment on the sensors, platforms and techniques in relation to soil survey. Indeed, the illustrative plates have captions dealing with vegetation, relief and land use, rather than soil conditions or soil boundaries. Some plates are in colour.

A wider selection of references would have been appropriate in a volume of this kind. For example, the work of Beckett, Evans, Mitchell and Western would suggest some of the pathways towards greater use of remote sensing.

The line diagrams are good but there are some typographical errors such as incorrect naming of the general editors on the dust jacket.

L. F. Curtis

L. F. Curtis is Reader in Geography at the University of Bristol, UK.

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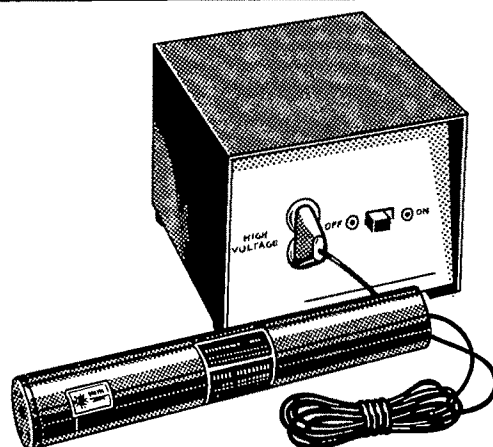
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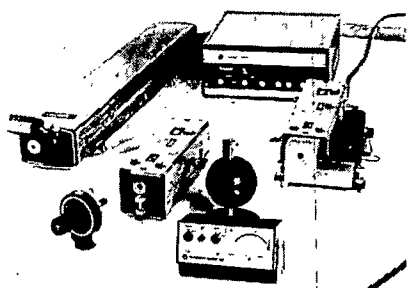
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what's new—lasers, optics, holography

These notes, prepared from material provided by the manufacturers, are intended to give an outline of the range of products on the market. More detailed information may be obtained by circling the appropriate numbers on the Reader Enquiry Card, bound inside the cover. A feature on centrifuges and rotary evaporators will appear in the 16 February issue.

Coherent. The Coherent Supergraphite series of argon and krypton ion lasers comprises 13 different models with 40 different configurations, providing multiline output power of from 800 mW to over 18 W TEM₀₀. With the ultraviolet option these systems will deliver up to 3.0 W guaranteed in the ultraviolet. Coherent krypton lasers have guaranteed TEM₀₀ output as high as 4.6 W in red wavelengths 3.0 W in the violet and 2.0 W in the ultraviolet. The Coherent CR series of dye lasers features a crystalline quartz birefringent filter which combines extremely low optical loss with precise tuning. Linewidth is resettable and the whole system is designed for ease of use. Optical components are mounted in a 2-inch diameter Invar rod for maximum amplitude and frequency stability. The CR 599 scanning dye laser system incorporates active closed-loop stabilisation to provide narrow linewidth and stable operation. The Coherent picosecond pulse package is based on Coherent ion and dye lasers and offers controlled pulse width and peak power, while the picosecond pulse picker ensures repetition rates. The new Model 307 'Noise Eater' can stand alone in front of any laser and give a

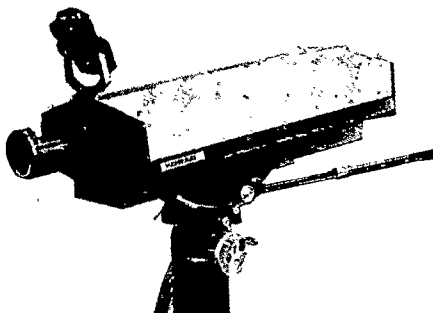


Laser equipment from Coherent

reduction of 40 db/d.c. and 26 db/1 MHz. Other Coherent equipment available includes collimators and lenses, mode lockers, interferometers, modulators, power meters and optics.

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Korad. Korad manufacture holographic laser systems for both reflection and transmission holography. Features include long coherence lengths, excellent wavelength repeatability, and availability as single and double pulse systems. The Korad mobile holocamera is completely self-contained and weighs less than 200 lb. The holocamera can accommodate any of the more than 20 different laser systems now available. The Pockels cell Q-switch KQS2 and KQS2DP laser



Korad KHCI holocamera

systems provide a precisely controlled laser pulse and are particularly suited to applications involving plasma and shock phenomena. Korad offer an extensive range of holographic accessories; these include alignment and reconstruction systems, the KA autocollimator, the KD1 photodetector, KDG delay generator and the 99/102C energy measurement system.

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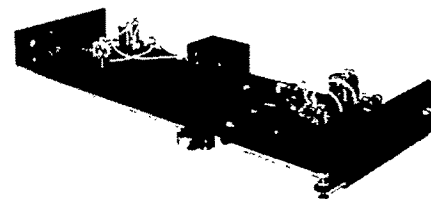
Hartley Measurements. The model 215G grating-tuned TEA CO₂ laser is a compact high energy density system: the 2-J multimode output has a gain switched spike only 40 ns wide, providing a peak power in excess of 15 MW. For TEM operation 0.7 J can be obtained in a 6-mm diameter beam of 3 milliradians divergence. Repetition rate is continuously adjustable from 0.2 to 2 ps. Manual push button/single shot firing are also available as standard. Full optics are provided with the unit and tuning is by means of a grating of 150 lines per mm. The unit is completely self-contained with a built-in power supply and all controls mounted in the laser housing.

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J K Lasers produce the System 2000 range of pulsed solid-state laser equipment. An updated set of data is available to explain the system, and

applications covered include holography, phytochemistry, plasma diagnostics, welding and drilling. The J K Lasers range of pulsed ruby lasers designed for use in holography has been extended to include a 10-J single-mode system. This high output energy has been achieved by two-stage amplification of a well-proven holographic oscillator. The system is capable of taking holograms of objects within a 5-m cube. As 1 J of the output energy can be used as the reference beam, large-format holograms of up to 1 m² can be made from a single pulse. J K Lasers will shortly be offering a range of standard dye lasers suitable for pumping with their solid state lasers. These models will be available with a full range of dyes, operating throughout the spectral range 400–800 nm.

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J K Lasers 2000 pulsed ruby laser

Electro-Photonics. The Electro-Photonics range includes a comprehensive range of flashlamp pumped dye laser systems, wavelength tunable across the entire visible spectrum, with options of mode-locking to produce picosecond laser pulses and second harmonic generation to provide tunable laser radiation in the ultraviolet. One interesting application is a new technique of 'rapid optical sampling of relaxation phenomena, involving two time-correlated picosecond pulse trains'. Two flashlamp-pumped, mode-locked and optically coupled dye lasers produce trains of picosecond pulses with definite, but variable time correlation. One pulse train is used to bleach the sample, while the other probes the decay of its transient transmission. Relaxation times between 15 ps and 1 ns can be monitored by one simultaneous activation of the two lasers that is, a single shot technique. In previous experiments each point of the relaxation decay curve was measured by splitting one laser pulse train and mechanically varying the delay between

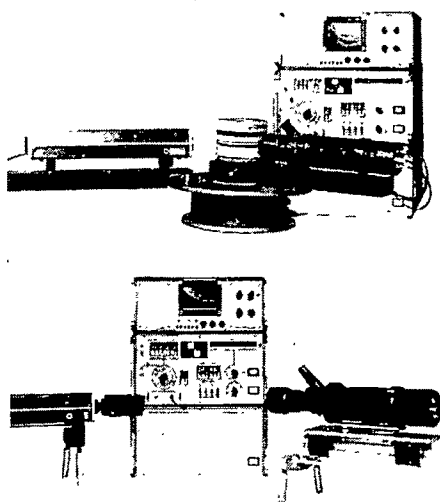
excitation and probe pulses between laser firings. There was also an added restriction that transmission measurements were limited to the probe wavelength. In this new technique, the probe wavelength can be tuned over a considerable wavelength range. Electro-Photonics also have developed systems for remote sensing of atmospheric pollution, in particular SO_2 and NO_2 . The technique used is differential absorption. The Electro-Photonics range includes the Model 23 high repetition rate dye laser, the Model 33 mode-locked dye laser and the Model 43 high-energy dye laser.

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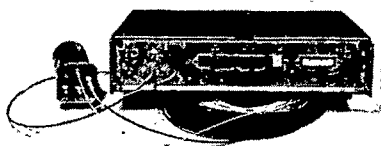
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Malvern 4300 (top) and 6200⁺ systems



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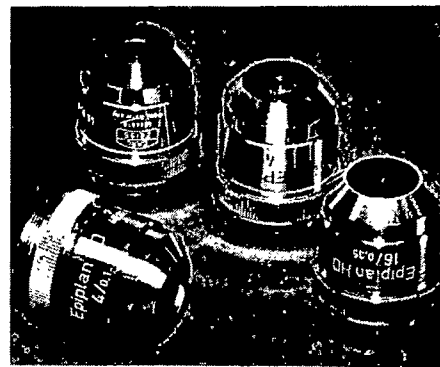
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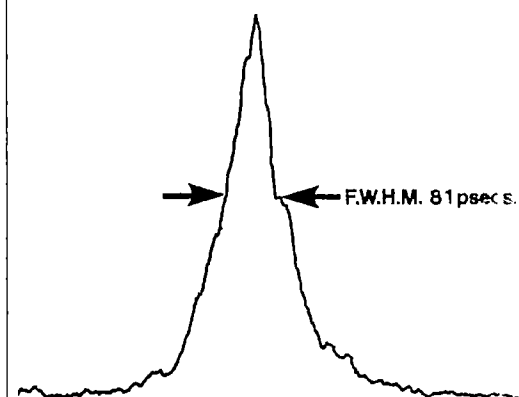
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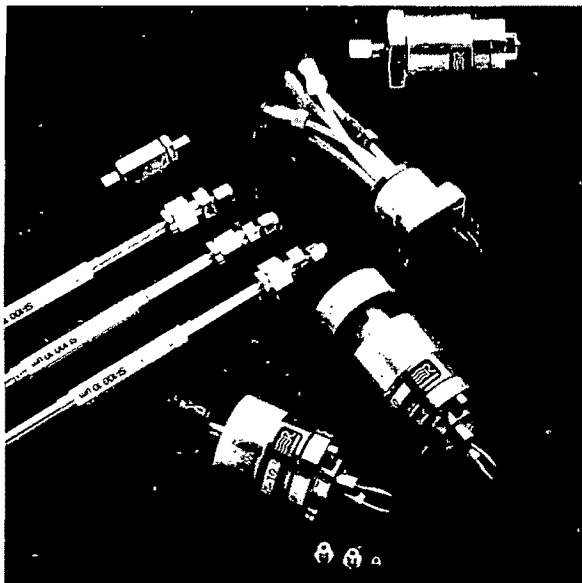
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For those who are seeking a change in their careers we now offer this new service...

APPOINTMENTS WANTED

will appear weekly; the advertising charges are £1.50 or \$3.00 per line (approximately seven words). Minimum 3 lines. Box numbers are automatically given without further charge, all correspondence to:

NATURE CLASSIFIED
4 LITTLE ESSEX STREET,
LONDON WC2R 3LF.
Telephone: 01-240 1101

RESOURCE Scientist, Ph.D., 37, seeking 1 to 3 yr., mgt./teaching/research position overseas. Background of contract research and science advisory in topics of water quality, marine resources and coastal land use planning and in areas of policy analysis, impact assessment and resource mgt. Nine years varied experience in field, lab. and office modes. Résumé, reports and papers available. Reply to: D. Zoelner, 5322 41st St., N.W., Washington, D.C. 20015. 1103(B)

AMERICAN Zoologist/Ethologist. Ph.D. (1974). 14 publications. French and German National Postdoctoral Fellowships. Five years university teaching. Four years admin. and consultant exp. (U.S. Naval Officer, G.E. Co.). Conversant Eng., Fr., Ger., It., Grk. Seeks teaching, research, or interesting alternative position after June 1978. Box No. 1047(B).

INORGANIC Chemist, aged 35, 36 publications, recently redundant in closing teachers training College seeks position U.K. or overseas. Box No. 1046(B).

APPOINTMENTS VACANT

UNIVERSITY OF SYDNEY LECTURESHIP IN ORGANIC CHEMISTRY

Applicants must have research interests in a mainstream area of Organic Chemistry.

The position is permanent but may be filled for three years in the first instance with possibility of permanency during that time, or in certain cases return fares. Appointee should preferably be able to commence duties in July 1978.

Salary range: SA14,851 to SA19,551 p.a.

Applications including curriculum vitae, list of publications and names of three referees by March 20, 1978, to the Registrar, University of Sydney, NSW 2006, Australia. Enquiries to Professor S. Sternhell, School of Chemistry, in the University. Conditions of appointment available from Association of Commonwealth Universities (Apsus), 36 Gordon Square, London WC1H 0PF. 1119(A)

FACULTY OPENING IN BIOCHEMISTRY University of Illinois Champaign-Urbana

The Department of Biochemistry is seeking applicants for a tenure track appointment at the level of Assistant Professor or higher to begin August, 1978. Applicants should be qualified to teach biochemistry courses at undergraduate and graduate levels. Only candidates who have demonstrated excellence in research and who are prepared to establish an active research program will be considered, preference will be given to those who have received graduate and postdoctoral training in strong research environments. The minimum salary at the Assistant Professor level is \$15,000 for a nine-month appointment; salaries at higher ranks are negotiable. For full consideration, application materials, including a curriculum vitae, a list of publications, a brief description of research interests and three letters of reference should be sent by February 1, 1978 to:

Professor Lowell P. Hager, Head
Department of Biochemistry
University of Illinois
Urbana, Illinois 61801
Telephone: (277) 333-3945.
The University of Illinois is an
Affirmative Action/Equal Opportunity Employer. 836(A)

UNIVERSITY OF BATH SCHOOL OF PHARMACY AND PHARMACOLOGY

Applications are invited for the following posts:

LECTURER IN PHARMACEUTICS (Ref. 77/158)

This appointment offers opportunity for a suitably qualified and experienced pharmacist to participate in the teaching and established research programme in the field of Pharmaceutics.

LECTURER IN PHYTOCHEMISTRY (Ref. 77/159) N

Applicants should be appropriately qualified and have experience in the chemistry of biologically active natural products.

Salary scale (under review) is £3,333 to £6,655; the starting salary will be set in the lower part of the scale.

Application forms and further particulars are obtainable from the Personnel Officer, University of Bath, Bath BA2 7AY. Please quote appropriate reference number. Closing date for applications: February 16, 1978. 1074(A)

UNIVERSITY OF JORDAN

Faculty positions: in Electrical Machines, Electronics and Electromagnetics available 1978. Doctorate and strong credentials are required. Responsibilities will include teaching, setting-up Laboratories and research. Rank and salary will be based on qualifications. Please send résumé with full information to the Dean, Faculty of Engineering and Technology, Jordan University, Amman, Jordan. 1044(A)

University of Bristol

LONG ASHTON RESEARCH STATION

Head of Crop Protection Division

The research staff is to be reorganised into four Divisions; the one concerned with chemical and biological crop protection will include fifty-five Science Group staff in varied disciplines. The work will concentrate, initially, on the principles of fungicidal activity, but will also comprise the strategy of crop protection, its ecological consequences and the practicability of biological control. Host and pest/pathogen combinations to be studied will include those suited to other work in the Station, and to plant pests and diseases important in the agriculture of South West Britain; collaborative work with other agricultural research institutions will be an important feature.

Appointment will be in the Senior Principal Scientific Officer grade (£8,858 to £10,006 per annum, including current pay supplements). Non-contributory superannuation scheme.

Applicants must have a good honours degree or equivalent in a relevant discipline, and considerable experience in the chemistry or biology of crop protection. As the post requires inspiring direction of staff expert in varied disciplines, and ability in scientific administration, great importance will be attached to personality, experience and the ability to foster collaborative work within and beyond the Division.

Further particulars from the Secretary, Long Ashton Research Station, Long Ashton, Bristol BS18 9AF, to whom applications together with the names of three referees should be sent not later than February 10, 1978. 1035(A)

UNIVERSITY OF LONDON

READERSHIP IN STATISTICS at

Imperial College of Science and Technology

The Senate invites applications for the above Readership tenable in the Department of Mathematics at the College.

The Reader will be required to have substantial research experience in theoretical or applied statistics or in applied probability theory or in the mathematical and probabilistic aspects of operational research. Supervision of postgraduate students will be an important part of the duties, as will lecturing on statistics to students of mathematics and engineering. The salary scale is £6,443 to £7,951 plus £450 London Allowance.

Applications (10 copies) must be received not later than February 15, 1978 by the Academic Registrar, (N) University of London, Senate House, London WC1E 7HU, from whom further particulars should first be obtained. 1102(A)

THE DISTILLERS COMPANY LIMITED FERMENTATION BIOCHEMIST

The Yeast and Food Division of the Distillers Company Limited requires a graduate to work in their small scale Pilot Plant at Glenochil Technical Centre, Menstrie, Clackmannanshire. Studies carried out in this Pilot Plant include yeast strain selection work, process development and examination of raw materials.

Applicants, who should preferably be under 28 years old, must have a degree in microbiology, biochemistry or closely related subjects together with relevant postgraduate experience. One or two years commercial or research experience with yeast would be an advantage.

Further details together with an application form can be obtained by writing to:

The Yeast Research Manager,
Yeast and Food Division,
The Distillers Company Limited,
Glenochil Technical Centre,
MENSTRIE,
Clackmannanshire FK11 7ES.
1016(A)

Experimental Officers

-Physical Chemistry

We have vacancies for two Experimental Officers in the Physical Methods Section of Research Department. The first is in a group undertaking kinetic and mechanistic studies for process development and stability evaluation of candidate drugs. The second is in a group investigating compounds' physico-chemical properties in relation to drug designs.

The candidates for both vacancies should be recently qualified (under 26), possess a degree or equivalent, have a special interest in physical organic chemistry and be familiar with analytical methods e.g. UV spectroscopy, potentiometry, HPLC etc. They should be able to combine precise experimental technique with judgement of data whilst remaining under the overall guidance of a Senior Chemist.

Previous experience in reaction kinetics

or knowledge of enzyme structure and mechanism would be helpful for the respective vacancies but is not essential.

Our modern research laboratories are situated in pleasant surroundings but within easy reach of main road and rail routes. Conditions of service, salary and assistance to married staff in moving house are designed to attract and retain staff of high calibre.

Please write giving details of qualifications and experience to:

Mr. M.J. Higgins, Personnel Officer
Imperial Chemical Industries Limited



Pharmaceuticals
Division
Meresside Research
Laboratories
Alderley Park
Nr. Macclesfield
Cheshire

1059(A)

THE ROYAL VETERINARY COLLEGE

University of London

Division of Preclinical Studies

DEPARTMENT OF ANATOMY

Applications are invited for the post of
LECTURER IN ANATOMY

The post becomes vacant on September 1, 1978, and is based at Camden Town. The person appointed will be expected to undertake teaching of the anatomy of large animals and will be expected to engage in research.

SALARY SCALE (under review): £3,805 to £7,105 including London Allowance. Initial salary determined by qualifications and experience. Superannuation under the Universities' scheme. Removal expenses up to £650 may be reimbursed in approved circumstances.

Application form and further details from the Assistant Secretary (Personnel), The Royal Veterinary College, Royal College Street, London NW1 0TU. Telephone: 01-387 2898.

CLOSING DATE FOR APPLICATIONS: February 24, 1978. 1125(A)

UNIVERSITY OF QUEENSLAND

Australia

**LECTURER IN
CROP SCIENCE**

DEPARTMENT OF
AGRICULTURE

To lecture in various aspects of crop (field, plantation or horticultural) production. Higher degree in Agricultural Science, or Science with agricultural experience. Demonstrated research capability in some aspect such as Crop Ecology, Crop Physiology or Cropping systems. March 6, 1978.

Salary: \$A14,632 to \$A19,262 per annum. Other Benefits: Superannuation, housing assistance, study leave, travelling and removal expenses.

Additional information and application forms are obtainable from the Association of Commonwealth Universities (Appts), 36 Gordon Square, London WC1H 0PF. 1085(A)

THE SCHOOL OF PHARMACY UNIVERSITY OF LONDON LECTURESHIP AND TEACHING FELLOWSHIP IN PHARMACOLOGY

Applications are invited for both these appointments effective at or about September 1, 1978 from suitably qualified persons.

For the Lectureship, postdoctoral teaching and research experience is required and an interest in Psychopharmacology, Cardiovascular Pharmacology or Endocrinology would be welcome.

For the Teaching Fellowship, which is of limited term, postgraduate research experience is required except for medical graduates.

Normal non-clinical university academic terms of appointment and salary scale. Further information can be obtained from Professor D. W. Straughan.

Applications to be submitted by February 6, 1978 in the form of letter accompanied by a C.V. and the names of two referees, to: The Personnel Officer, (N) The School of Pharmacy, 29/39 Brunswick Square, London WC1N 1AX. 1034(A)

NATIONAL INSTITUTE OF AGRICULTURAL BOTANY REGIONAL TRIALS OFFICERS

Applications are invited for two appointments of Regional Trials Officers at N.I.A.B. Regional Trials Centres situated at:

(a) School of Agriculture, Sutton Bonington, Loughborough, Leics. Ref. SV/247/N;

(b) A.D.A.S. Experimental Husbandry Farm, Preston Wynne, Hereford. Ref. SV/250/N.

Candidates should possess an honours degree in Agriculture, Agricultural Botany, Botany or Plant Sciences; preference will be given to those who are 26 or over with at least three years postgraduate experience in agriculture or field experimentation.

Salary according to age, qualifications and experience on Grade III—£3,816 to £6,298 p.a. including supplements.

Further particulars and application forms from the Establishments Officer, National Institute of Agricultural Botany, Huntingdon Road, Cambridge CB3 0LE. Telephone: Cambridge 76381 ext 234. Closing date: February 13, 1978. 1112(A)

UNIVERSITY OF RHODESIA FACULTY OF ENGINEERING

LECTURESHIP VACANCIES

The faculty of Engineering has recently been established to provide four-year Honours degrees in Civil, Electrical, Mechanical and Interdisciplinary Engineering, with G.C.E. A-level entry. The Faculty buildings and facilities are fully operative, most professors and lecturing staff are in post, and during 1977, the teaching of the first classes of final (fourth) year students was undertaken.

Applications are invited now for **Lecturer/Senior Lecturer** posts, in the following subject areas. (In each discipline candidates with wide experience and interests will be at an advantage. Areas of special need are shown in brackets).

(a) Civil Engineering: (Geotechnology, Water Engineering, Surveying and Transportation)

(b) Electronic and Power Engineering: (Control Systems, Electromagnetics, Materials)

(c) Mechanical Engineering: (Thermodynamics, Heat Transfer, Solid Mechanics, Fluid Mechanics, Mechanical Design)

(d) Materials Science: (Interdepartmental specialist appointment).

Duties include teaching, research, and assistance with administration.

Minimum qualifications: Good Honours degree (higher degree preferred), plus substantial postgraduate experience. Corporate membership of a professional Engineering Institution desirable.

Salary Scales (Approx. equiv. stg.)
Senior Lecturer £7,352 by £278 to £7,630 by £288 to £7,918 by £298 to £9,706;

Lecturer Grade I £6,675 by £248 to £7,667;

Lecturer Grade II £4,118 by £218 to £5,208 by £248 to £6,448.

Starting salary according to qualifications and experience.

Subject to University permission extra earnings to a specified limit for consultancy may be retained in full. Above this limit they are shared with the University.

Appointments: These may be on permanent pensionable terms or for a fixed term of 1 or 2 years in the first instance. Family passages and allowance towards transport of effects.

One and two-year Contracts: non-pensionable salary as above, plus 10%. Unless born in Rhodesia, expatriates on Contract are exempted from military service. Good rented accommodation is available, some of it fully furnished, close to good, inexpensive Junior and Senior schools and the University Medical Aid Scheme.

Permanent Pensionable Terms: Triennial Contact Visits and Sabbatical Leave with assisted passages overseas; superannuation and medical aid schemes; rented accommodation available for first three years. Installation grant and loan.

Applications: One copy giving personal particulars (including full names, place and date of birth), qualifications, fields of special interest, experience and publications, and giving names and addresses of three referees, should be sent by **February 28, 1978** to: The Senior Assistant Registrar (Engineering), University of Rhodesia, P.O. Box MP. 167, Mount Pleasant, Salisbury, Rhodesia, from whom further particulars may be obtained. Overseas applicants should send an additional copy to the Association of Commonwealth Universities (Appts), 36, Gordon Square, London WC1H 0PF, from whom further particulars may also be obtained. Applicants should state the earliest date available and also the preferred date.

British subjects considering applying for posts in Rhodesia are urged to consult the Foreign and Commonwealth Office (telephone 233-4143) or their nearest British Consular Office.

1064(A)

Pharmacologist

At Fisons we have already established a reputation in the field of allergy research. We are now setting up a small new team to do further innovative research in this area.

We require a Graduate Pharmacologist who would join as a senior member of the new team. If you have a PhD, or are a graduate with a few years' experience, and you have an interest in immunopharmacology then phone Mr P Sheard (0509 66361) for further information, or write to Mr A B Johnston, Fisons Ltd, Pharmaceutical Division, Bakewell Road, Loughborough, Leicestershire, for an application form quoting reference RD1.



1114(A)

UNIVERSITY OF
RHODESIA

FACULTY OF SCIENCE

Applications are invited from suitably qualified persons for the post of:

FACULTY BIOMETRICIAN

Appointment will be at the level of Senior Lecturer or Lecturer. The appointee will be based in the Department of Agriculture and will be required to teach undergraduate courses in Biometry to students of Agriculture and the Biological Sciences. The Biometrician also provides a consulting service for staff and research students in the Faculty.

Salary Scales (Approx. Stg. equiv.):

Senior Lecturer £7,352 by £278 to £7,630 by £288 to £7,918 by £298 to £9,706.

Lecturer Grade I £6,675 by £248 to £7,667.

Lecturer Grade II £4,118 by £218 to £5,208 by £248 to £6,448.

Both permanent pensionable terms and short-term one- or two-year contracts are offered.

Permanent Pensionable Terms: Family passages and allowance towards transport of effects on appointment. Installation loan of up to half of one year's salary if required. Unfurnished University accommodation guaranteed for a period of at least three years for persons recruited from outside Rhodesia. Sabbatical leave and triennial contact visits with travel allowance. Superannuation and medical aid schemes.

Short-term Contracts: Family passages and allowances towards transport of effects. Assistance with accommodation for persons recruited from outside Rhodesia.

Applications: (6 copies) giving full personal particulars (including full name, place and date of birth, etc.), qualifications, experience and publications and names and addresses of three referees, should be submitted by February 15, 1978 to the Assistant Registrar (Science), University of Rhodesia, P.O. Box MP 167, Mount Pleasant, Salisbury, Rhodesia, from whom further particulars may be obtained. Overseas applicants should send a copy of their applications to Association of Commonwealth Universities (Appts), 36 Gordon Square, London WC1H 0PF, from whom further particulars may also be obtained.

British subjects considering applying for posts in Rhodesia are urged to consult the Foreign and Commonwealth Office (telephone 233-4143) or their nearest British Consular Office. 1067(A)

UNIVERSITY
OF MELBOURNE
LECTURER
(Limited Tenure)
DEPARTMENT OF
PHYSICAL CHEMISTRY

Applicants with experience in any field of physical chemistry will be considered. Preference may be given to applicants with experience in the application of statistical mechanics to water and aqueous solutions, polyelectrolytes, electrode processes or surfaces. Current research areas within the Department include colloid and surface chemistry, radiation and photochemistry, picosecond spectroscopy, polymer chemistry, vibrational spectroscopy, thermodynamic properties of solutions, electrochemistry and reaction kinetics.

SALARY: \$A14,851 to \$A19,551 per annum.

Further information including details of application procedure and conditions of appointment is available from bourn, Parkville, Victoria, 3052. The Registrar, University of Melbourne, Australia, or from the Association of Commonwealth Universities (Appts), 36 Gordon Square, London WC1H 0PF. Applications referring to Position Number 612 003 should be addressed to The Registrar and close on March 1, 1978. 1118(A)

CSIRO

AUSTRALIA

Research Scientist

Centre for Animal Research
and Development
Bogor, Indonesia

CSIRO has a broad charter for research into primary and secondary industry areas. The Organisation has approximately 7,000 employees—2,300 of whom are research and professional scientists—located in Divisions and Sections throughout Australia.

Field: Nutritional Biochemistry (Poultry)

General: The Centre, situated at Ciawi near Bogor in West Java, is a joint activity of the Australian Development Assistance Bureau (ADAB), the Indonesian Department of Agriculture and CSIRO. Its establishment phase will be completed in mid-1978 when the construction of facilities is complete. Currently, it has a staff of about 25 Australians and 200 Indonesians. Total staff, when the Centre is fully operational, is expected to be about 300, including 50 professional scientists.

The research program is broadly aimed at increasing the productivity of the Indonesian livestock industries to meet the increasing demand for food of animal origin. The research will be concerned with the nutrition, reproduction, health and other aspects of the husbandry of chickens, ducks, cattle, buffalo, sheep, goats and pigs.

Supporting the research effort are analytical and veterinary services, mainly concerned respectively with feedstuffs analysis and disease screening and diagnosis for the Centre's experimental livestock.

Duties: A senior scientist is required to lead a research group which is developing nutritionally balanced and economically attractive feedstuffs for the Indonesian poultry industry in collaboration with other research scientists working in this field.

Qualifications: Ph.D. degree in an appropriate field, or equivalent, together with relevant research experience.

Salary: Salary will be the subject of negotiation and will be in the ranges of Senior Research Scientist/Principal Research Scientist: A\$18,171—A\$24,528 p.a. Appointment at a higher level would be considered for a quite outstanding applicant. Overseas service allowances are also payable.

Tenure: 3 years with possibility of extension.

Further information: Further information on the Centre is available on request from the Secretary, CSIRO, P.O. Box 225, Dickson, A.C.T. 2602, Australia.

Applications (in duplicate), stating *FULL* personal and professional details, the names and addresses of at least two professional referees, and quoting reference number 1000/34 should reach:—

The Personnel Officer, Australian Scientific Liaison Office, Canberra House, Maltravers Street, LONDON WC2R 3EH by 17th FEBRUARY 1978.

Applications in U.S.A. and Canada should be sent to:—

The Counsellor (Scientific), Embassy of Australia, 1601 Massachusetts Avenue, N.W., WASHINGTON D.C. 20036 U.S.A. 1111(A)

UNIVERSITY OF
GLASGOW
CHEMISTRY DEPARTMENT
(GC-MS UNIT)

Applications are invited for a one-year postdoctoral appointment supported by the Boots Co. Ltd. for a study of the metabolism of a new drug in man. The project will involve open-tubular gas chromatography combined with mass spectrometry, based on LKB9000 and Dupont 21-490F (EI/C1) instruments. Candidates should have experience in gas chromatography and preferably also in GC-MS.

Salary range £3,333 to £3,761 with U.S.S. benefits.

Applications, naming two referees, should be sent to Professor C. J. W. Brooks, Chemistry Department, University of Glasgow G12 8QQ.

In reply please quote Ref. No. 4038M. 1055(A)

UNIVERSITY OF KEELE
VICE-CHANCELLOR

The Council and the Senate of the University of Keele wish to appoint a Vice-Chancellor to succeed Professor W. A. C. Stewart, who will retire on September 30, 1979. Applicants are invited to write in confidence to the Lord Rochester (Pro-Chancellor and Chairman of the Joint Committee of Council and Senate to consider the appointment), c/o the Registry, University of Keele, not later than March 31.

Suggestions on persons suitable for consideration would also be welcome.

The University Council reserves the right to appoint to the post by invitation.

Information about the University and further particulars of the post may be obtained from the Registrar, The University, Keele, Staffordshire ST5 5BG. 1101(A)

UNIVERSITY OF
MELBOURNE
CHAIR OF INORGANIC
CHEMISTRY
(Re-advertised)

The Chair of Inorganic Chemistry, which is one of the three Chairs in the School of Chemistry, is vacant after the appointment of Professor D. R. Stranks as Vice-Chancellor of the University of Adelaide.

Salary: \$A31,248 per annum.

Further information, including details of application procedure, superannuation, travel and removal expenses, housing assistance, and conditions of appointment is available from the Association of Commonwealth Universities (Appts), 36 Gordon Square, London WC1H 0PF.

Applications close on March 31, 1978. 1066(A)

A senior overseas position in Horticultural Research for agricultural development in MALAWI £8,300 (part tax free)

As Assistant Principal Agricultural Research Officer at the Hvumbwe Research Station you would conduct procedures affecting agricultural development in this progressive Central African country.

This would be concerned with the production of either vegetables or fruits such as citrus, guavas, mangoes, etc. or tree nuts such as tung and macadamia.

The station provides initial planting material for distribution to farmers, recommends production methods and assists official bodies on produce exporting and markets.

You should have a B.Sc. in Agriculture, Biology, or Botany and have at least 4 years' research experience in the field of agronomy, including horticultural research.

The benefits of low tax and living costs in Malawi can be well worthwhile. In addition, the salary has a tax-free element of £5 682. A terminal gratuity of 25% of total basic salary over the contract term of 30-36 months is also payable tax-free.

Other overseas benefits include free passages, settlement allowances, subsidised accommodation, generous home leave, medical care, etc.

Malawi has a warm sunny climate and friendly people. Its natural scenic beauty contrasts with the modern social and leisure facilities of its expanding cities and townships.

Write for details and application form—quoting Ref. No. MHCL/21, before the closing date 2nd February, 1978—to: 1033(A)

Malawi Malawi High Commissioner,
Recruitment Section,
33 Grosvenor Street, London, W1.

RADCLIFFE INFIRMARY OXFORD

Full-time Research Assistant/Technician required to work in a unit investigating beta cell function in Diabetes. Some experience in histology or animals would be useful but not essential.

Whitley Council scales and conditions. Research grant for 2 years.

Contact Dr R. C. Turner, Department of the Regius Professor of Medicine, Radcliffe Infirmary, Oxford. 1030(A)

CHARLES COULSON SUMMER SCHOOL IN THEORETICAL CHEMISTRY Oxford University from September 10-23, 1978.

Further details from Prof. N. H. March, Theoretical Chemistry Department, 1 South Parks Road, Oxford OX1 3TG. Closing date for applications. May 31, 1978. 1090(A)

LINCOLN COLLEGE (University College of Agriculture) New Zealand LECTURER IN BIOCHEMISTRY

The Council of Lincoln College invites applications for the position of Lecturer in Biochemistry.

Applicants should have a degree in Science or Agricultural Science, and a postgraduate qualification. Preference will be given to those with experience in Agricultural Biochemistry. Duties will include teaching Biochemistry in the course for Bachelor of Agricultural Science, and the appointee will be expected to carry out research in Biochemistry related to Agriculture.

Commencing salary according to qualifications and experience within the range NZ\$10,239 to \$12,543 per annum. At present salaries are supplemented by an 'Interim Special Allowance' of 3.5%.

Travel and removal expenses reimbursed up to specified limits. New Zealand Government Superannuation available.

Conditions of Appointment are obtainable from the Association of Commonwealth Universities, (Appts), 36, Gordon Square, London WC1H 0PF, or from the Registrar of the College.

Applications close on **March 6, 1978.** 1076(A)

THE UNIVERSITY OF MANCHESTER INSTITUTE OF SCIENCE AND TECHNOLOGY RESEARCH ASSISTANT (BIOCHEMISTRY)

A vacancy exists for a Research Assistantship in Biochemistry (male or female) to work on cell mechanisms associated with the regulation and growth and maturation of nervous connections during nervous system development. Previous research experience with tubulin or microtubules or with membrane bound receptors would be an advantage but is not essential. The position is suitable either as a postdoctoral appointment or alternatively for someone wishing to pursue a research programme for a Ph.D. Appointment will be for two years in the first instance. Salary will be on Range 1B (£2,904 to £4,190 per annum), or Range 1A (£3,333 to £5,627 per annum).

Applications, quoting reference 00/8/AI and accompanied by a curriculum vitae and names and addresses of two referees, should be sent to the Registrar, UMIST, P.O. Box 88, Manchester M60 1QD by January 31, 1978. 1049(A)

INFORMATION RESEARCH OFFICER

The Metal Society has been awarded a grant by the British Library to carry out a review of scientific and technical information services in the field of metals and requires a Research Officer for the project.

Basic qualifications required are a knowledge of metals and metals information services and an awareness of the latest techniques for information handling.

The appointment will be for a period of three years: a starting salary up to £5,000 p.a. will be negotiated.

For an application form and for further information write to:

John Vaughan
The Metals Society
1 Carlton House Terrace
London SW1Y 5DB
marking your envelope

**Confidential:
Information Research Officer**

The closing date for receipt of completed applications will be February 24, 1978. 1113(A)

university of wales university college of swansea

Senior Research Assistant

Applications are invited for the vacancy of Senior Research Assistant in the Department of Zoology to work with Dr N. A. Ratcliffe on an N.E.R.C. sponsored project on invertebrate host defence mechanisms and the influence of environmental parameters on these reactions. Candidates should be near to completing or have completed a Ph.D. in Marine Microbiology, Cell Biology or Immunology and should have experience in the use of radioisotopes and basic microbiological techniques. A knowledge of cell separation and fractionation, ecology and/or histochemistry would also be advantageous.

The appointment, which will date from March 1, 1978, or as soon as possible thereafter, will be for one year in the first instance, with the possibility of renewal for a further two years. The commencing salary will be on a scale up to £3,761 per annum plus U.S.S./U.S.D.P.S. benefits.

Further particulars and application forms (2 copies) may be obtained from the Personnel Officer, University College of Swansea, Singleton Park, Swansea SA2 8PP, to whom they should be returned by **Friday, February 10, 1978.** 1124(A)

PORTSMOUTH POLYTECHNIC Biophysics Group SENIOR TECHNICIAN POST 677

The successful candidate will be responsible for the running and maintenance of a Bruker WH 270 MHz plus NMR spectrometer. The spectrometer is used by a large research group working on the structure of chromatin and chromosomal proteins. An interest in and an ability to take part in this research work would be an advantage.

Salary Scale: T3/4 £2,922 to £3,702 plus £473.70 to £512.70 per annum supplement.

Application forms and further details are available from the Staff Officer, Portsmouth Polytechnic, Alexandra House, Museum Road, Portsmouth or by telephoning Portsmouth 27681 Ext. 317. Closing date Monday, January 30, 1978. 1037(A)

QUEEN ELIZABETH COLLEGE

(University of London)

Campden Hill Road, Kensington, London W8 7AH PHYSIOLOGY DEPARTMENT TECHNICIAN GRADE 4

A vacancy exists for a grade 4 Technician to join a group serving the needs of both teaching and research within the Physiology Department. Candidates should possess O.N.C. equivalent or higher qualifications, and experience in either teaching or research laboratory work would be an advantage.

Salary, including London weighting within range £3,420 (plus L.W. supplement £21.00) to £3,867 (plus L.W. supplement £23.00).

Contact Ms G. M. Dorman for application form: Tel.: 01-937 5411 ext. 499. 1068(A)

THE GRASSLAND
RESEARCH INSTITUTE
Hurley, Maidenhead, Berks.
PLANT PHYSIOLOGIST

A Plant Physiologist is required in the Botany Department to join a small team, led by the Head of Department, investigating the physiology of forage crops in the field. The aim is to provide a rational basis for the improvement of forage production systems by understanding how their productivity is determined by their underlying physiology, and the way management, including method of harvesting, environment and differences in genotype bring about their effect.

Particular emphasis has been placed on crop gas-exchange using an enclosure method. Carbon 14 labelling is also being used. Experience with infra-red gas analysis and radioisotope techniques and other relevant techniques would be an advantage. A sound knowledge of plant physiology and the ability to handle and interpret data are, however, more important.

The Institute is grant aided by the Agricultural Research Council and is an associated Institution of the University of Reading.

Qualifications:- First or upper second class honours in Botany or allied subject.

The appointment will be in the grade of Scientific Officer or Higher Scientific Officer, point of entry depending on qualifications and experience.

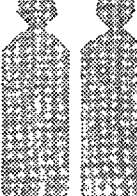
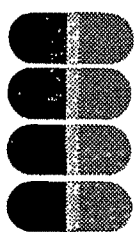
Salary:- Scientific Officer £2,592 to £4,032 p.a. including phase I and II pay supplements.

Higher Scientific Officer £3,745 to £4,976 p.a. including phase I and II pay supplements.

At least 2 years postgraduate experience is normally required for appointments to the higher grade. There is a non-contributory superannuation scheme.

Applications to the Secretary by January 31, quoting 3/G/36.

1109(A)



Scientific Officer

Drug Metabolism

Ware

A vacancy has occurred in our Biochemical Pharmacology Department for a biochemist to investigate the metabolism of potential new drugs using both *in vitro* and *in vivo* systems.

Applicants should either be recently qualified Ph.D. graduates with experience of metabolic biochemistry, or good honours graduates with equivalent experience.

The work is conducted in a creative atmosphere in modern laboratories in a pleasant countryside location in Hertfordshire.

The position carries a competitive salary which will reflect the age and experience of the successful candidate. Other benefits include pension and profit sharing schemes, and assistance with relocation expenses where appropriate.

Apply to:

Dr. I. Collins, Personnel Manager, Allen & Hanburys Research Ltd., Ware, Herts.
SG12 0DJ. Telephone Ware 3232.



Allen & Hanburys Limited



1121(A)

Wageningen

The Agricultural University of Wageningen (Netherlands) invites applications for appointment as

Full Professor in Nature Conservation and Nature Management

(due to the imminent retirement of Prof. Dr. M. F. Mörzer Bruyns). Candidates should be able to fulfill the following conditions:

1. substantial experience in the field of scientific backgrounds and applications of Nature Conservation and Nature Management.
2. Qualifications in the field of teaching, education and organisation on an academic level.
3. Willingness and capability for
 - a. effectuation, stimulation and coordination of research.
 - b. making contributions to interdisciplinary activities within and outside the University.
 - c. guiding the integrated approach, necessary for the subject in case.
4. Willingness to cooperate in activities issuing from the Law of University Reform (W.U.B.).
5. Willingness and capability to learn the Dutch language within a brief period; familiarity with French is also highly desirable.

Candidates for this position and those who want to recommend suitable and available persons are asked to contact the "Griffie van de Faculteit der Landbouwwetenschappen", Herenstraat 25, Wageningen, Netherlands within 4 weeks, mentioning all relevant particulars. Letters should be marked "nr. 77-146" on the envelope. Further information may be obtained from the "Griffie" on request.

1083(A)

UNIVERSITY OF SOUTHAMPTON MEDICAL ONCOLOGY (Cell Biologist) Lectureship (or Temporary Lectureship)

Applications are invited for the post of Lecturer (or Temporary Lecturer) in the Professional Medical Oncology Unit. The successful candidate will play an active role in the research activities of the Unit, and will be involved in the day to day running of the laboratories. The ideal candidate will be a cell biologist with an interest in cell membranes and preferably some biochemical experience. Salary scale £3,333 to £6,655 (under review), initial salary will depend upon qualifications and experience.

Further particulars may be obtained from Mr C. W. L. Swann, The University, Southampton SO9 5NH to whom applications (7 copies from United Kingdom applicants) should be sent not later than February 3, 1978. Please quote ref: N/870/A.

1075(A)

MEMORIAL UNIVERSITY OF NEWFOUNDLAND Canada

DEPARTMENT OF BIOLOGY FISHERIES BIOLOGIST

Applications are invited for a faculty position (rank open), with primary responsibility in the development of a graduate programme (M.Sc. and Ph.D.) in the field of fisheries biology. Preference will be given to candidates with an established background and practical experience in applied fisheries research. Applicants should submit a full curriculum vitae and the names of three referees to Dr G. Robin South, Head, Department of Biology, Memorial University of Newfoundland, St John's, Newfoundland, Canada A1B 3X9 by March 20, 1978.

1063(A)

THE GOVERNMENT OF THE SYRIAN ARAB REPUBLIC ALEPPO UNIVERSITY ALEPPO UNIVERSITY HOSPITAL

Invites applicants for posts in Anaesthesia vacant 1977/1978. Qualifications: F.R.C.S. onc. In addition to five years experience.

Salary: £8,000 to £10,000 per annum; plus £1,350 to £1,700 accommodation allowance per annum.

—One month paid holiday per annum.

—Return tickets to country of residence at the end of contract, also for wife and two children.

—Contract for one year renewable. Please write for full details to:

The Rector
University of Aleppo
Syrian Arab Republic

864(A)

TECHNICIAN required in the DIVISION OF ENTERIC PATHOGENS Colindale, London NW9

Suitable qualifications would include a relevant science degree, F.I.M.L.S., A.I.M.L.S., or H.N.C. in Medical Laboratory Sciences with experience in microbiology. The laboratory studies enterobacterial ecology on a national and international scale and is also concerned with transferable drug resistance in the enterobacteria.

Salary according to age and experience, but not less than £2,184 plus £354 per annum London Weighting, plus £454.50 pay supplements 1 and 2.

Applications with the names of at least two referees to Personnel Officer, Central Public Health Laboratory, Colindale Avenue, London NW9 5HT. Tel. 01-205 7041. 1053(A)



CENTRAL ELECTRICITY RESEARCH LABORATORIES

Kelvin Avenue, Leatherhead, Surrey, KT22 7SE.

is expanding its research on the environmental aspects of electricity generation. The following posts are now available:

TERRESTRIAL ECOLOGISTS

With a higher degree in botanical, agricultural or soil sciences or forestry, to undertake field and experimental studies of plant/soil interactions with atmospheric pollutants. Based at the above location.

Post 1 PROJECT LEADER

With relevant experience and proven ability to lead a small team. Appointment within a salary range rising to £7550 or exceptionally £8350, inclusive of supplements.

Post 2 MEMBER OF TEAM

Appointment within salary ranges rising to £5500 or £6400, inclusive of supplements.

MARINE ECOLOGISTS

Based initially at the CERL Marine Biology Laboratory, Fawley, Nr. Southampton.

POST 3 MEMBER OF TEAM

With postgraduate experience in marine biology, to work on the effects on marine organisms of entrainment in cooling water systems. Appointment within salary ranges rising to £5500 or £6400, inclusive of supplements.

POST 4 MEMBER OF TEAM

With postgraduate experience in ecological modelling, to apply modelling techniques to questions of power station siting. Appointment within salary ranges rising to £5500 or £6400, inclusive of supplements.

Applications, quoting reference RL/75, on standard forms which may be obtained from and returned to the Head of Personnel Services at the above address. Closing date is: 11th February 1978. Application forms may be obtained by telephoning Leatherhead 74488 Extension 329.

1079(A)

UNIVERSITY OF NOTTINGHAM DEPARTMENT OF ELECTRICAL AND ELECTRONIC ENGINEERING

Applications are invited for a lectureship in solid state electronics which will be filled from the earliest possible date. Initial salary within the first three points of the lecturers' scale: £3,333, £3,547, £3,761.

Applications and further particulars from the Staff Appointments Officer, University of Nottingham, University Park, Nottingham. Closing date February 3, 1978. Ref: 541. 1107(A)

THE CITY UNIVERSITY DEPARTMENT OF CHEMISTRY

RESEARCH ASSISTANT

required to work on the kinetics of oxygen reduction on semi-conducting oxide/graphite electrode. The appointment will be for one year initially. The research is supported by the Science Research Council. Candidates should have a good honours degree in Chemistry or Materials Science. Salary will be on the scale £2,927 to £4,199 p.a. plus £450 p.a. London Allowance.

Applications, together with the names of two referees should be sent to Dr A. C. C. Tseung, Department of Chemistry, The City University, St John Street, London EC1V 4PB. 1089(A)

UNIVERSITY OF BATH SCHOOL OF BIOLOGICAL SCIENCES LECTURER IN HORTICULTURE

Applications are invited for a lectureship in the Horticulture Group of the School. Candidates should have a degree in Horticulture, postgraduate experience, and preferably a Ph.D. An interest in agronomy (particularly vegetable production) is essential.

Starting salary will be set in the lower part of the range £3,333 to £6,655 p.a.

Application forms and further particulars available from the Personnel Officer, University of Bath, Bath BA2 7AY, quoting reference number 77/154 N. Closing date for applications February 20, 1978. 1073(A)

The University of Sheffield DEPARTMENT OF CHEMISTRY POSTDOCTORAL ASSISTANT

Applications are invited for the post of postdoctoral Assistant in an investigation of the synthesis of model enzyme systems related to chorismate synthetase. Tenable up to two years commencing as soon as possible and supported by S.R.C. Salary in the range £3,333 to £3,547 on Range IA with superannuation. Applications with the names of 2 referees should be made to Dr E. Haslam, Department of Chemistry, University of Sheffield, Sheffield S3 7HF. Quote Ref. R.60/G. 988(A)

KENYATTA UNIVERSITY COLLEGE (A constituent College of the University of Nairobi)

Applications are invited for the post of
LECTURER

in the Botany Department

Candidates should have a Ph.D. in Plant Biochemistry or Plant Physiology, with several years University teaching experience. The appointee is expected to teach courses in Plant Biochemistry and Physiology and also conduct research in his/her area of specialisation. Experience in a teacher training institution will be an added advantage. Salary (at present under review) Kf1,800 to Kf3,096 p.a. (Kf1=£1.35 sterling). The British Government may supplement salaries in the range £3,354 to £3,618 p.a. (sterling) for married appointees or £2,184 to £2,400 p.a. (sterling) for single appointees (reviewed annually and normally free of all tax) and provide childrens' education allowances and holiday visit passages. Terms of service include subsidised housing, membership of S.S.S.F. or F.S.S.U. and a non-contributory medical scheme, and family passages. Detailed applications (2 copies) including a curriculum vitae and naming 3 referees should be sent by airmail by February 17, 1978 to the Registrar, Kenyatta University College, P.O. Box 43844, Nairobi, Kenya. Applicants resident in the U.K. should also send one copy to the Inter-University Council, 90/91 Tottenham Court Road, London W1P 0DT. Further particulars are available from either address.

1116(A)

PROFESSOR DEPARTMENT OF MOLECULAR BIOLOGY UNIVERSITY OF GENEVA

Applications should be made before February 28, 1978 to, and information can be obtained from:

Secrétariat de la faculté des sciences de l'université de Genève, quai Ernest Ansermet 20, CH 1211 GENEVE 4 (Switzerland). 1082(A)

MEDICAL RESEARCH COUNCIL UNIT OF CLINICAL PHARMACOLOGY UNIVERSITY DEPARTMENT OF CLINICAL PHARMACOLOGY RADCLIFFE INFIRMARY OXFORD OX2 6HE

Technician required to assist in a research project concerned with the effect of disease and drugs on enzyme activities. Applicants should have H.N.C., degree or equivalent qualifications and be familiar with general biochemical techniques. Experience in enzymology or biochemical pharmacology would be a distinct advantage. Salary will be in the range £2,823 to £4,050 (including supplements), depending upon age and qualifications. Applications with the names of two referees should be sent to the Chief Technician at the above address by January 31. 1058(A)

GUY'S HOSPITAL MEDICAL SCHOOL DEPARTMENT OF MEDICINE TECHNICIAN

required for one year in the first instance to work on a project funded by the Arthritis and Rheumatism Council on the immune response to onco-fetal antigens in rheumatoid arthritis. Salary for qualified Technician not less than £2,982 plus £354 London Weighting. Experience in immunological techniques essential. Starting date April 1. Applications, with the names of two referees, to be sent to the Secretary, Guy's Hospital Medical School, London Bridge SE1 9RT, quoting Ref: D.M.2.

1050(A)

UNIVERSITY OF MELBOURNE CHAIR OF OPTOMETRY

Applications are invited for appointment to the newly established Chair of Optometry.

Salary: \$A31,248 per annum. Further information, including details of application procedure, superannuation, travel and removal expenses, housing assistance, and conditions of appointment, is available from the Association of Commonwealth Universities (Appts), 36 Gordon Square, London WC1H 0PF. Applications close on March 31, 1978. 1069(A)

I.L.R.A.D.

International Laboratory for Research on Animal Diseases invites applications from qualified candidates for the following position:—

IMMUNO-CHEMIST

I.L.R.A.D. is recruiting for a core or visiting scientist's position in Immuno-chemistry for immediate employment. I.L.R.A.D. is a modern research institution located in Nairobi, Kenya, and has an international staff. The Laboratory is conducting research on trypanosomiasis and theileriosis, two important diseases that constrain livestock production for food and other uses in many developing countries. Research at I.L.R.A.D. offer the opportunity to work on meaningful problems with international implications, to conduct investigations at headquarters in Nairobi and elsewhere and to relate to a group of scientists carrying out multi-disciplinary activities of broad scope.

QUALIFICATIONS:

Minimum 3 years postdoctoral (or equivalent) experience. Solid experience with current techniques in molecular immunology (protein purification, detection and characterisation of cell surface antigens, radioimmunoassay, antigen-antibody interactions, lectin technology, gel technology, antibody function etc.). The candidate should also be familiar with and conversant in several aspects of cellular immunology. Scientists interested in being considered for this post should provide a current curriculum vitae (including a synopsis of training, research experience and publications). Three referees who can be contacted in support of their candidacy should also be included.

TERMS

Salary—Negotiable

Other include Housing allowance, commutation allowance, pension scheme and medical aid scheme, etc.

This information should be sent to:—

The Director,
I.L.R.A.D.,
P.O. Box 30709,
NAIROBI,
Kenya to reach him not later than February 6, 1978. 1110(A)

Technical Services

**TECHNICIAN
(HORTICULTURE)**

Salary: £2,529 to £2,853

(plus supplement range: £454.08 to £470.28)

To carry out horticultural duties and give general assistance in the running of Rumleigh Experimental Station particularly the growing and propagation of plants in the open and under glass for teaching and research projects.

The Experimental Station is situated at Bere Alston, 14 miles from the Polytechnic (City Centre).

Applicants should possess a current driving licence, an O.N.C. in Horticulture or equivalent and at least 3 years' appropriate experience.

Application forms, to be returned by February 10, 1978, can be obtained with further particulars from the Personnel Officer. 1072(A)

**NATIONAL VEGETABLE
RESEARCH STATION
SOIL SCIENTIST**

A SOIL SCIENTIST is required for studies on the soil aspects of site to site variations in yield of field vegetables, the work involving the development of theories and quantitative models for differences in productivity between soils and testing these in field experiments. The appointment will be in the Grade of Higher Scientific Officer (£3,745 to £4,976) or Senior Scientific Officer (£4,707 to £6,300) including supplements.

Non-contributory superannuation scheme.

First or Upper Second Class Honours Degree. At least two years research in Agronomy, Soil Science or other relevant scientific subject is required for appointment as H.S.O.; for appointment as S.S.O. evidence will be required of the candidate's ability to carry out research at this level, preferably by reference to published papers. An adequate mathematical background is essential.

Full particulars and application form from the Secretary, National Vegetable Research Station, Wellesbourne, Warwick. Closing date for receipt of applications is February 9, 1978. 1038(A)

**RESEARCH ASSOCIATE
IN ATMOSPHERIC
SCIENCE (AERONOMY)—**

The Department of Atmospheric and Oceanic Science of the University of Michigan has a post-doctoral research position available in the area of terrestrial and planetary aeronomy. This position involves analysis and interpretation of data obtained from a variety of space missions (e.g. Pioneer-Venus, Voyager, Dynamics Explorer, etc.). This work is to be carried out in collaboration with Professors T. M. Donahue and A. F. Nagy. Send resumes to: University of Michigan, Office of Professional and Administrative Staff Services, 1020 "NN" L. S. and A. Building, Ann Arbor, Michigan 48109. A non-discriminatory, affirmative action employer. 1095(A)

**MEDICAL RESEARCH
COUNCIL
TOXICOLOGY UNIT**

Junior Technician or young Technician required to assist in biochemical laboratory systems of mammalian liver. Qualifications: O.N.C. (Applied Biology) or equivalent for Junior grade or H.N.C. (or equivalent) for Technician grade. Salary within the range of £1,571 to £2,298 plus London Weighting and Supplements or £2,511 to £3,738 plus London Weighting and Supplements. Grade and salary according to age, qualifications and experience (preference given to candidates with experience in biochemical techniques).

Apply in writing to The Director, Toxicology Unit, Medical Research Council Laboratories, Woodmansterne Road, Carshalton, Surrey SM5 4EF. 1057(A)

**THE UNIVERSITY OF
MANCHESTER
RESEARCH ASSOCIATES
IN GERIATRIC MEDICINE**

There are vacancies for an Electron Microscopist (appointment for up to 3 years) and a Mechanical Engineer (appointment for up to 2 years). These appointments, preferably post-doctoral, are as part of a new team developing research into the aging bladder and the problems of incontinence. Salary range: £3,333 to £5,015 p.a. The work will be carried out in the research laboratories of the Department of Geriatric Medicine at the University Hospital of South Manchester, Nell Lane, Manchester M20 8LR, under the overall direction of Professor J. C. Brocklehurst, from whom further details may be obtained. 1060(A)

**UNIVERSITY OF LONDON
Canterbury Hall of Residence**

The University requires to fill the post of

BURSAR

at this Hall of Residence for 222 women students, becoming vacant owing to retirement on August 1, 1978.

Candidates should possess appropriate qualifications and experience preferably in the running of a University Hall of Residence or similar establishment.

Salary is on the scale £3,182 by £204 to £4,160 (subject to review) plus £450 London Allowance and free board and residence.

Further particulars are available from Mrs Debbie Croucher, Personnel Section, Room 223, University of London, Senate House, Malet Street, London WC1E 7HU. Closing date for receipt of applications is February 17, 1978. 1117(A)

Hydrologist

for resource data in MALAWI

up to £7,865 (part tax free)

This interesting position calls for a Hydrological Data Processing specialist, to handle all water resource data as part of the national Development Programme of this progressive Central African country.

You would be responsible to the Principal Hydrologist for

- supervising and developing an existing DP system
- training DP staff
- publishing annual data in acceptable international format
- reviewing and assessing incoming hydrological data
- updating storage/retrieval of past data
- analysing data for water resource projects

You should have a degree in Mathematics, Civil Engineering, Geography or a related discipline, plus a sound knowledge of hydro-meteorological practices. At least 3 years' experience of modern DP methods—using COBOL and/or FORTRAN—is essential and experience of ICL job control language would be advantageous.

The salary has a tax-free element of £5,532. A terminal gratuity of 25% of total basic salary over the 30-36 months contract is also payable tax free.

Other overseas benefits include free passages, settlement allowances, subsidised accommodation, generous home leave, medical care, etc.

Malawi has a warm sunny climate, a friendly people and its natural scenic beauty contrasts with the modern social and leisure facilities of its expanding cities and townships.

Write for details and application form—quoting Ref. No. MHC/10, before the closing date 2nd February, 1978—to:

1031(A)

Malawi Malawi High Commissioner,
Recruitment Section,
33 Grosvenor Street, London, W1.

**UNIVERSITY OF
ABERDEEN****SENIOR LECTURER IN
AGRICULTURAL
BIOCHEMISTRY
(Animal Nutrition or
Biochemistry)**

Applications are invited for the above University post in the School of Agriculture. Candidates should have an honours degree in Biochemistry, Agricultural Biochemistry or Chemistry, and substantial post-graduate research experience in either Animal Nutrition or Animal Biochemistry.

Salary on scale £6,443 to £7,951 per annum, with appropriate placing.

Further particulars from The Secretary, The University, Aberdeen, with whom applications (2 copies) should be lodged by February 11, 1978. 1987(A)

**DEPARTMENT OF PHYSICS
ASSISTANT
PROFESSORSHIP**

Subject to adequate funding, a position may become available July 1, 1978 in one of the following fields

**QUANTUM OPTICS
BIOPHYSICS**

Salary depends on qualifications. The closing date for applications is March 31, 1978.

Exceptional candidates in other fields may be considered.

All correspondence should be addressed to:

Professor Luis de Sobrino,

Chairman,

Appointments Committee,

Department of Physics,

The University of British

Columbia,

2075 Westbrook Place,

Vancouver, British Columbia,

Canada V6T 1W5. 823(A)

THE UNIVERSITY OF BRITISH COLUMBIA

In anticipation of increased activity in coal exploration, production and utilization within the Province of British Columbia, the University of British Columbia, a government funded institution which provides instruction and research opportunities to 23,000 students, is expanding its coal activities. A new coal research laboratory is scheduled for completion in 1978 and the following faculty appointments are expected to be made in August, 1978.

Within the Department of Mineral Engineering:

Coal Processing—Working in conjunction with three mineral processing faculty members, the appointee can expect to lead the coal processing activities of the department by providing appropriate instruction and research direction to undergraduate and graduate students.

Applicants are expected to be holders of advanced degrees who, through participation in coal-processing plant design and operation, and/or coal processing research, are well versed in current technology.

Mine Services—In collaboration with three mining engineer faculty members, the appointee will engage in the teaching and research aspects of mine ventilation, materials handling systems, and the control of dust and noxious gases. The appointee will be a graduate engineer who, through appropriate underground mining experience, is conversant with modern practice in the specific fields mentioned above. Coal mining experience is a decided asset for this appointment.

Rock Mechanics—The appointee will hold an appropriate advanced degree and will be oriented to the measurements of in-situ rock stress distributions and the design of underground openings in strata-bound sequences. In cooperation with three mining engineer faculty members, the appointee will be responsible for undergraduate instruction and graduate studies in applied rock mechanics. Currently the department possesses a variety of rock properties measurement equipment and has direct access to an extensive computer facility.

Within the Department of Geological Sciences:

Coal Geologists—The department is seeking a geologist who will develop a coal geology program that integrates with the coal activities in Mineral Engineering. The appointee will be involved in teaching coal geology, the supervision of graduate students, and a research program in geologic aspects concerned with mine development, such as structure, stratigraphy correlation, petrology, and geo-statistics. A Ph.D., in hand or pending, and field experience in coal geology are essential. It is expected the appointment will be made at the Assistant of Associate level.

Applicable salary ranges are as follows:

Assistant Professor	\$20,000—\$27,000 per annum
Associate Professor	\$27,000—\$32,000 per annum
Full Professor	\$30,000—\$35,000 per annum

Enquiries, or applications accompanied by a full curriculum vitae and the names of three referees, should be directed as follows:

a) for positions within Mineral Engineering

The Head,
Department of Mineral Engineering,
University of British Columbia,
VANCOUVER, B. C., Canada V6T 1W5

b) for the position within Geological Sciences

Dr. W. H. Matthews
Chairman, Coal Committee
Department of Geological Sciences
University of British Columbia,
VANCOUVER, B. C., Canada

This announcement should not be construed as a commitment by U.B.C. to make appointments in rigid adherence to the above descriptions. 926(A)

INSTITUTE OF CHILD HEALTH NEONATAL DEPARTMENT POSTDOCTORAL RESEARCH ASSISTANT IN BIOCHEMISTRY

Applications are invited for the post of Research Assistant to take part in a new project on control of fetal lung development. The person appointed will continue and extend current studies on the cellular composition and biochemical maturation of pathological and experimental lung tissue. Applicants should have postgraduate laboratory experience and will be expected to hold a Ph.D. or M.Sc.. The appointment will be for 3 years.

Starting salary in the range £3,805 to £4,649 including London Weighting.

Full details and application forms available from Dr J. S. Wigglesworth, Department of Paediatrics and Neonatal Medicine, Hammersmith Hospital, Du Cane Road, London W12 0HS. 1108(A)

THE UNIVERSITY OF LEEDS DEPARTMENT OF EARTH SCIENCES RESEARCH FELLOW

A vacancy exists in the above Department for a

with Ph.D. and with experience of high pressure/temperature apparatus and techniques, to determine experimentally carbonate equilibria in the system $\text{CaCO}_3\text{-MgCO}_3\text{-FeCO}_3\text{-MnCO}_3$ under typical metamorphic conditions.

The appointment is for a fixed term of two years from May 1, 1978 or as soon as possible thereafter. Salary on the IA Scale for Research and Analogous Staff £3,761 to £3,975 (under review). Applications naming three referees should be addressed to Professor J. C. Briden, Department of Earth Sciences, The University, Leeds LS2 9JT, from whom further particulars may be obtained, as soon as possible and in any case not later than March 31, 1978. 1097(A)

THE UNIVERSITY OF MANCHESTER DEPARTMENT OF MEDICAL BIOPHYSICS RESEARCH ASSOCIATE IN WOLFSON IMAGE ANALYSIS UNIT

Applications are invited for this post in a group developing image analysis techniques for application to problems in clinical medicine, medical research and industry. The work involves not only a considerable intellectual challenge but also close collaboration with industry to produce image analysis solutions in marketable form.

Candidates should have research or development experience and probably a physical science or mathematical background. The project aims at exploiting MAGISCAN, a computer based image analysis system. This means that computer programming will be involved but a wider problem solving ability is sought.

The post is tenable initially for up to two years. Initial annual salary range: £3,333 to £5,015. Superannuation.

Applications should be made by January 31, Dr C. J. Taylor, Department of Medical Biophysics, Stopford Building, The University, Manchester M13 9PT. 1061(A)

UNIVERSITY OF BIRMINGHAM DEPARTMENT OF SOCIAL MEDICINE

A graduate is required to take part in an epidemiological study of multiple primary tumours at Birmingham and West Midlands Regional Cancer Registry. Applications are invited from biologists with experience in statistical methods with a knowledge of Fortran programming, but other graduates with suitable experience would be considered.

The appointment is on the Research Associate IB grade (£2,904 to £4,190). Initial salary will depend on age and experience.

Applications should be sent to The Assistant Registrar, The Medical School, Birmingham B15 2TH, by February 10, 1978. 1071(A)

UNIVERSITY OF NOTTINGHAM DEPARTMENT OF ELECTRICAL AND ELECTRONIC ENGINEERING Applications are invited for a LECTURESHIP

in solid state electronics which will be filled from the earliest possible date. Initial salary within the first three points of the lecturers' scale: £3,333, £3,547, £3,761.

Applications and further particulars from the Staff Appointments Officer, University of Nottingham, University Park, Nottingham.

Closing date: February 3, 1978. Ref: 541. 1122(A)

UNIVERSITY OF WOLLONGONG Australia

SENIOR LECTURER/LECTURER
IN COMPUTING SCIENCE (A)
(Tenurable Appointment)
LECTURER IN COMPUTING
SCIENCE (B)
(Limited Term Appointment
of up to 4 Years)

Applicants should have a higher degree in Computer Science and some experience in research and/or teaching. The successful applicants will participate in the development of the Computing Science course program at undergraduate and graduate level. It is anticipated, subject to the approval by the Council of the University, that Computing Science activities (currently part of the Mathematics Department) will form the Department of Computing Science in the near future.

Currently active research areas are: portability of operating systems (UNIX in particular); interactive languages; databases and text searching algorithms. A Computing Science laboratory with 8 terminals time sharing an Interdata 7/32 computer is available for teaching and research.

Further information may be obtained from Professor J. Reinfelds, in the University.

Commencing salary according to qualifications and experience, will be within one of the ranges:—Senior lecturer \$A19,676 to \$A22,955; Lecturer \$A14,632 to \$A19,262.

For details of appointment write to the Bursar, The University of Wollongong, Box 1144, P.O. Wollongong, N.S.W., 2500, Australia (please specify position A or B or both), or from the Association of Commonwealth Universities (Appts), 36 Gordon Square, London WC1H 0PF.

Closing date March 1, 1978. 1070(A)

DEPARTMENT OF NUTRITION AND FOOD SCIENCE MASSACHUSETTS INSTITUTE OF TECHNOLOGY FACULTY POSITION

The Department, a large multi-disciplinary teaching and research unit with activities in all principal areas of nutrition and food science is seeking an applicant to fill the following faculty position:

An M.D., with research training and preferably with a Ph.D. degree, and experience and a major interest in human and clinical nutrition. The appointee will be expected to teach graduate level courses concerned with human and clinical nutrition, to develop a vigorous research programme and become actively involved in the research programmes of the M.I.T. Clinical Research Center.

The appointment will be at the Assistant or Associate Professor rank depending upon the qualification of the successful applicant.

Curriculum vitae, together with representative publications and the names of five referees should be submitted before February 1, 1978 to: Dr V. R. Young, Chairman Search Committee, Room 56-331, Massachusetts Institute of Technology, Cambridge M.A. 02139, U.S.A.

M.I.T. is an Equal Opportunity Employer. Representatives of minority groups are urged to apply. 830(A)

PHARMACEUTICAL INDUSTRY IN PARIS, FRANCE

seek

AN EXPERIENCED PHYSIOLOGIST

The position contains mainly experimental brain surgery and physiology in the cat and the small monkey.

Please send your response to:

E.P.R.I.
126 Bld Auguste Blanqui
75013 PARIS
référence no. 446.

1086(A)

UNIVERSITY OF ALABAMA IN BIRMINGHAM

Medical Center, U.S.A.

Microbiology

The Department of Microbiology, Schools of Medicine and Dentistry, with the Diabetes Research and Training Center and the Institute of Dental Research, respectively, invite applications from individuals with research interests in:

1. Regulation of Viral Protein Synthesis
2. Oral Microbiology — Immunology.

Candidates should have a Ph.D. or equivalent terminal degree, expect to develop a strong research program, and participate in the education of graduate and professional students. A curriculum vitae, relevant reprints, description of research interests, and three letters of reference should be forwarded to: Dr Kenneth J. Roozen, Vice-Chairman, Department of Microbiology, The University of Alabama in Birmingham, Birmingham, Alabama 35294, U.S.A., not later than March 1, 1978. An affirmative action/equal opportunity employer. 1100(A)

ST BARTHOLOMEW'S HOSPITAL

London EC1A 7BE

RESEARCH ASSISTANT/ TECHNICIAN

DEPARTMENT OF
MEDICAL MICROBIOLOGY

Applications are invited from Technicians and Graduates (M.Sc./Ph.D.) for a position available for 1 year in the first instance, but with a possibility of renewal for a further 2 years, to work on a research project on the serology of anaerobic bacteria and relationship to pathogenicity in diagnostic medical microbiology. Applicants with training and/or experience in serological techniques, microbiology and/or immunology will be considered. The work will involve the preparation and purification of microbial antigens, preparation and purification of antisera and immunofluorescent techniques.

Salary according to experience on the scale £3,333 to £5,627 plus London weighting plus earning supplements.

Applications stating age, qualifications, present post, past experience, and the names of two referees, should be sent to the Personnel Department within three weeks of the date of publication, quoting reference RP 90/77.

City & Hackney Health District—part of The City & East London AHA(T). 1052(A)

SEARCH FOR EXTRASOLAR PLANETS

An anticipated new grant includes funds available as of February 1978 for a research associate who would participate in a search for low-mass companions of solar-type stars using a recently constructed Digicon radial velocity meter with Fabry-Perot wavelength calibration. Duties of the position will include telescopic observations, their reductions and interpretation, and a continuous improving of the instrument and computer reductions to minimise the errors of radial and rotational velocities. The candidate must have a doctorate and experience with computer programming, advanced instrumentation, and astrophysical observations. Please send resume and three letters of recommendation from recognised astronomers to Dr K. Serkowski, Lunar and Planetary Laboratory, University of Arizona, Tucson, Arizona 85721. An equal opportunity/Affirmative Action/Title IX, Section 504 Employer. 1094(A)

UNIVERSITY OF NEWCASTLE UPON TYNE

MICROBIOLOGICAL CHEMISTRY
RESEARCH LABORATORY

POSTDOCTORAL RESEARCH ASSOCIATES

Applications are invited for two posts of Postdoctoral Research Associates in a group studying the biosynthesis and function of teichoic acids in bacterial cell walls and membranes. The work includes either structural or metabolic aspects in this rapidly developing field and experience in either biochemistry or microbiology would be appropriate.

The appointments are for three years Salary in range 1A £3,333 to £5,627 per annum, plus superannuation benefits. Tenable from February 1, 1978 or by arrangement. Applications with curriculum vitae and the names of two referees to Professor Sir James Baddiley, F.R.S., Microbiological Chemistry Research Laboratory, The University, Newcastle upon Tyne NE1 7RU. 1062(A)

FRESHWATER BIOLOGICAL ASSOCIATION

River Laboratory

ZOOLOGIST

required to assist in team project on Biological Classification of Rivers. Experience in river invertebrate taxonomy and ability to work methodically on samples essential, interest in computing desirable. Three-year contract equivalent to Scientific Officer in Civil Service. Gross salary within the range £2,592 to £4,032.

Full details and application form from the Secretary, Freshwater Biological Association, River Laboratory, East Stoke, Wareham, Dorset BH20 6BB. Closes February 10. 1081(A)

UNIVERSITY COLLEGE OF NORTH WALES

Applications are invited for the post of
LECTURER IN ZOOLOGY

The appointment will be on the scale: £3,333 to £6,655 per annum (under review).

Applications (two copies), giving full details of qualifications and experience, together with the names and addresses of three referees, should be sent to the Assistant Registrar (Personnel), University College of North Wales, Bangor, Gwynedd LL57 2DG, from whom further particulars may be obtained.

Closing date for the receipt of applications: February 28, 1978. 1123(A)

ELECTROCHEMIST

with experience in anodic synthesis and other preparative electrochemical reactions required for new project for chemical synthesis. Give full details of past experience in the above fields. Maybridge Chemical Co. Ltd., Tintagel, N. Cornwall. 1096(A)

AN FORAS TALUNTAIS

DAIRY AND FOOD PROCESSING RESEARCH

Applications are invited for two posts in the Dairy Technology Department, Moorepark Research Centre, Fermoy, Co. Cork. Research will be mainly concerned with Unit Process Evaluation and Development and the subsequent introduction of processes at Industrial Level.

ESSENTIAL: An Honours University degree, or equivalent, in Food or Dairy Engineering or other appropriate discipline.

DESIRABLE: Relevant research experience, particularly in dehydration or fermentation.

Commencing salary and level of appointment will depend on qualifications and experience. Marriage and Childrens' allowances. Non-contributory pension scheme and provision for Widows' and Orphans' pensions.

Application forms and further particulars may be obtained from The Personnel Department, An Foras Taluntais, Headquarters, 19 Sandymount Ave., Dublin, 4. (Tel: 688188). Latest date for receipt of completed application forms Friday, February 10, 1978. 1041(A)

Microscopist

Redland Technology serves the multi-national Redland Group of Companies which is concerned with the manufacture of building and construction materials.

A wide variety of interesting research and development projects are currently in progress at the New Technology and Product Development Centre at Horsham, where a vacancy exists for a person experienced in the use of optical and/or electron microscopes and in carrying out the associated specimen preparation techniques. Duties will include responsibility for the optical microscope facility and provision of a service to the technical staff at the centre and in the various manufacturing divisions.

Day release for further study and training schemes are in operation. Salary will be on a scale up to £4,000 depending on age, qualifications and experience. Please apply to Dr. S. E. Horsley, Redland Technology, Graylands, Horsham, W. Sussex. RH12 4QG. 1048(A)

Redland Technology

THE GOVERNMENT OF THE SYRIAN ARAB REPUBLIC ALEPPO UNIVERSITY

Faculty of Medicine

Invites applicants for the following posts as visiting professors for short periods (minimum 6 weeks) vacant 1977/1978 and 1978/1979.

Internal Medicine: M.R.C.P. or American Board in the following:

- Endocrinology
- Immunology
- Liver Diseases
- Infectious Diseases.

Surgery: F.R.C.S. or American Board in the following:

- Anatomy
- Pathology
- Surgical Anatomy
- Surgical Pathology.

Paediatrics: M.R.C.P. or American Board, in addition to five years experience.

£660 per month

£230 Accommodation allowance per month

Return tickets for the professor.

Please write for full details to:

The Rector
University of Aleppo
Syrian Arab Republic 865(A)

MEMORIAL UNIVERSITY OF NEWFOUNDLAND

Canada

DEPARTMENT OF
BIOCHEMISTRY

POSTDOCTORAL FELLOW PHYSICAL BIOCHEMISTRY

A postdoctoral position in the area of Physical Biochemistry of lipids in natural and artificial membranes and lung surfactant is available for one year effective immediately.

Salary—\$11,200 per annum. Send resume and names of three referees to: Dr K. M. Keough, Department of Biochemistry, Memorial University of Newfoundland, St John's, Newfoundland, Canada A1B 3X9. 1065(A)

LIVERPOOL POLYTECHNIC SCHOOL OF PHARMACY

LECTURER GRADE II

in the microbiological and sterilisation aspects of Dosage Form Design and Development. A higher degree and relevant teaching or industrial experience would be an advantage. Active research is expected and encouraged.

Salary scale: £3,744 to £5,985 p.a.

Application forms and further particulars are available from the Personnel Office, Liverpool Polytechnic, Richmond House, 1 Rumford Place, Liverpool L3 9RH. Tel: 051-227 5581. Ext. 43.

Please quote reference LP/113.

Closing date is fourteen days from the appearance of this advertisement. 1051(A)

CURATOR

THE GILBERT WHITE AND
OATES MUSEUMS

seek full- or part-time Curator. Details: The Wakes, Selborne, Hampshire. 1029(A)

MEMORIAL UNIVERSITY OF NEWFOUNDLAND DEPARTMENT OF GEOLOGY

The Department of Geology invites applications for three faculty positions in **Sedimentology and Marine Geology** with special reference to the Labrador continental margin (subject to the availability of funds).

One appointment will be a regular faculty position at a salary and rank commensurate with qualifications and experience. The appointee will direct and participate in the activities of a research group working in the following specialities: Mesozoic and Cenozoic clastic sedimentology, clay mineralogy, sedimentary geochemistry and micropaleontology. Applications from suitably qualified candidates with working experience in the petroleum industry will be especially welcome.

Two appointments will be at the rank of Assistant Professor (Research) and will be for a two-year term in the first instance. Applicants should have proven research ability in one (or more) of the specialist fields listed above.

Applications for all three positions, which must include a detailed curriculum vitae and the names and addresses of three referees, should be sent by March 31, 1978 to: Dr David Skevington, Head, Department of Geology, Memorial University of Newfoundland, St John's, Newfoundland, Canada A1B 3X5. 1077(A)

THE POLYTECHNIC OF CENTRAL LONDON

School of Engineering and Science
Science Division

LECTURER II— PHYSIOLOGY

Required for April 1978 or earlier if possible to teach mainly on the B.Sc. (C.N.A.A.) Modular Degree course in science. Applicants should have special interests in Endocrinology and Neurophysiology. Some knowledge of Immunology would be an advantage.

Salary: £4,146 to £6,387 which includes £402 London Allowance and supplement of between £444 and £492.

Details and application form (to be returned asap) from The Establishment Officer, P.C.L., 309 Regent Street, London W1R 8AL. 01-580 2020, Ext. 212. 1092(A)

ST GEORGE'S HOSPITAL MEDICAL SCHOOL (University of London)

Graduate research assistant or technician required to work with Professor M. J. Davies and Mr D. J. Parker on organ culture of human cardiac valves. The work may also involve some scanning electron microscopy. Previous experience with tissue or organ culture desirable but not essential. Salary up to £4,162 per annum.

Further details obtainable from Professor M. J. Davies, Department of Histopathology, St George's Hospital Medical School, Cranmer Terrace, London SW17 0RE. 1056(A)

Wanted and for Sale

Scientific and Technical Reviews
Please inquire for runs or single volumes

**SANTO VANASIA, 58 Via M.
Macchi, 20124 MILANO, Italy
XI(L)**

FRANK HORNE AWARDS

A Studentship and grants for research or study of seeds, plants or crops in relation to the agricultural and horticultural industry are available for 1978/79. Details and application forms from K. C. Batchelor, National Institute of Agricultural Botany, Huntingdon Road, Cambridge, England. 1099(F)

THE UNIVERSITY OF LEEDS DEPARTMENT OF RADIOTHERAPY

Applications are invited from graduates with good honours degree in biological sciences for a

POSTGRADUATE RESEARCH STUDENTSHIP

The project will involve a cellular kinetic and quantitative microscopical study of the action of drugs and radiation on experimental tumors.

Applications giving a full curriculum vitae, including the names and addresses of two academic referees, should be forwarded to Professor C. A. F. Joslin (Regional Radiotherapy Centre, Cookridge Hospital, Leeds LS16 6QB) from whom further details are available. 1040(F)

The Macaulay Institute for Soil Research, Aberdeen

RESEARCH STUDENTSHIPS

Applications are invited from students who hold or expect to hold upper second class honours degrees in the appropriate subject for Ph.D. studentships at the standard S.R.C./A.R.C. rates for work in the following areas:

Spectroscopy (E.S.R. and Laser)
Physical Chemistry (Sorption and Thermochemistry)
Organic Chemistry (Enzymes)
Plant Physiology (Calcium translocation)

Candidates, who must have British nationality, should submit applications to Professor T. S. West (Director), The Macaulay Institute for Soil Research, Craigiebuckler, Aberdeen AB9 2QJ. 987(F)

BRUNEL UNIVERSITY DEPARTMENT OF NON-METALLIC MATERIALS RESEARCH STUDENTSHIP

Applications are invited for a postgraduate research post tenable from March 1, 1978, for three years. The studentship is sponsored by the Blue Circle Group to study the development and the mechanical behaviour of Polymer Cements.

Interested persons with a good first degree in Materials or Polymer Science or other relevant discipline should apply to Dr K. A. Hodd, Department of Non-Metallic Materials, Brunel University, Uxbridge, Middlesex. 1126(F)

HERIOT-WATT UNIVERSITY DEPARTMENT OF BREWING AND BIOLOGICAL SCIENCES RESEARCH ASSOCIATESHIP

Applications are invited from graduates in microbiology for the post of Research Associate in the Department of Brewing and Biological Sciences, Heriot-Watt University. This is a joint project with the University of Newcastle upon Tyne. It is funded by the S.R.C. for a period of three years under the direction of Drs F. G. Priest and M. Goodfellow and involves a polyphasic taxonomic study of the genus *Bacillus*. The successful applicant may be able to register for a higher degree. The initial salary is £2,904 per annum (under review).

Informal enquiries may be made to Dr F. G. Priest (Tel. 031-225 8432 Ext. 307).

Application forms are available from the Staff Officer, Heriot-Watt University, Chambers Street, Edinburgh EH1 1HX to whom they should be returned by February 2, 1978. 1032(O)

UNIVERSITY OF OXFORD

Applications are invited from Biochemists, Geneticists and Zoologists for a three year postdoctoral assistantship with a group investigating the genetic control of collagen expression, particularly in somatic cell hybrids. The project, which is financed by the Medical Research Council, operates between the Department of Pathology, Radcliffe Infirmary, and the Genetics Laboratory of the Department of Biochemistry.

The salary range is £3,761 to £4,190 per annum. Closing date: February 28, 1978.

Curriculum vitae and the names of two referees should be sent to Dr B. Sykes, Department of Pathology, Radcliffe Infirmary, Oxford, from whom further details are available. 1115(P)

GRADUATE ASSISTANTSHIPS

are available for well qualified pre-doctoral students in Geophysical Fluid Dynamics, particularly in the area of computer and laboratory modelling of variations in the climate. A strong background in mathematics and physics is needed but prior training in a geophysical discipline is not necessary. Minorities and women are especially encouraged to apply. Write to Director, Geophysical Fluid Dynamics Institute, 18 Keen Building, Florida State University, Tallahassee, Florida 32306. 784(P)

THE UNIVERSITY OF BRITISH COLUMBIA DEPARTMENT OF CHEMISTRY GRADUATE TEACHING ASSISTANTSHIPS

Graduate Teaching Assistantships are available for candidates with high academic records who wish to pursue further studies towards the M.Sc., and Ph.D. degrees in chemistry. Excellent research facilities are available in all areas of modern chemical science, ranging from bio-inorganic chemistry to chemical physics. The minimum stipends including summer research assistantship will be \$6,120.00 per annum for first year and \$6,500.00 for students entering with a Master's Degree. The stipends are current under review. In addition, many special merit awards are made to those Graduate Teaching Assistants who show excellence in their performance of their duties. Numerous graduate scholarships are also available for students with outstanding academic records.

Application forms and further particulars may be obtained from the undersigned:

Professor C. A. McDowell,
Department of Chemistry,
The University of
British Columbia,
Canada V6T 1W5

773(P)

MASSEY UNIVERSITY

Palmerston North, New Zealand

POST DOCTORAL FELLOWSHIPS

Applications are invited for Postdoctoral Fellowships tenable in an Department of the University.

The academic activities of the University are grouped under the following faculties and schools.

Opportunities for study and research are available in the following field

AGRICULTURAL AND HORTICULTURAL SCIENCE

Agricultural economics and marketing, Farm Management, Agricultural Engineering, Agronomy, Animal Science (dairy, sheep, beef, pigs), Horticultural Science and Plant Health, Poultry Science, Soil Science.

BUSINESS ADMINISTRATION

Agricultural Business, Business Studies, Data Processing, Economic Financial Management and Accounting, Marketing, Personnel Administration

FOOD SCIENCE AND BIOTECHNOLOGY

Food Technology, Biotechnology (i.e. processing of Biological Materials Industrial Management and Engineering, Industrial Management and Industrial Mathematics.

HUMANITIES

English, History, French, German, Philosophy.

SCIENCE

Biochemistry, Biophysics, Botany, Chemistry, Genetics, Microbiology, Zoology.

SOCIAL SCIENCE: Applications from scholars in any of the usual Social Science Disciplines will be welcomed, but particular preference may be given to those interested in Educational Sociology, Human Geography and/or Planning, Mathematics, Social and Applied Psychology, and Economics.

VETERINARY SCIENCE

Physiology and Anatomy, Veterinary Pathology and Public Health Clinical Sciences.

Information concerning the research activities of the departments associated with these groups are given in the University Calendar. Interested applicants are advised to write to the Head of the appropriate department in the course of preparing an application.

A Fellowship will be tenable for one year, with possible extensions, and carries an emolument of NZ\$8,500. Travelling expenses up to NZ\$1,500 may be provided.

Further details of the position and of the University, together with conditions of appointment and information to be supplied by applicants, may be obtained from the Association of Commonwealth Universities (Apsu), Gordon Square, London WC1H 0PF, or from the Registrar of the University.

Applications close on **February 17, 1978.**

1120(E)

UNIVERSITY COLLEGE OF NORTH WALES, BANGOR

DEPARTMENT OF
BIOCHEMISTRY AND
SOIL SCIENCE

POSTDOCTORAL RESEARCH FELLOWSHIP PHYTOCHEMISTRY ECOLOGICAL

Applications are invited for a N.E.R.C. Postdoctoral Fellowship to work in collaboration with Dr R. G. Wyn Jones on the accumulation of organic solutes, particularly nitrogenous compounds in halophytic and xerophytic plants.

Experience in the isolation and characterisation of small molecular weight organic compounds and an interest in plant ecology and taxonomy will be highly desirable. The appointment is for two years and will commence on May 1, 1978, at a salary of £3,333 per annum plus superannuation.

Applications (two copies), together with the names and addresses of two referees should be sent to the Assistant Registrar (Personnel), University College of North Wales, Bangor, Gwynedd LL57 2DG, by February 25, 1978, from whom further details may also be obtained. 1088(E)

UNIVERSITY OF WARWICK

POSTDOCTORAL RESEARCH FELLOW

in

Biological Sciences

Applications are invited for a Postdoctoral Fellowship within the Chloroplast Development Research Group. This three-year post, supported by the S.R.C., is concerned with the study of photosynthetic membrane formation in plants, with special reference to the control of synthesis of the light-harvesting chlorophyll *a/b* binding protein by light. Experience with *in vitro* translation systems would be a distinct advantage. Initial salary will be up to £3,761 p.a. on the Range 1A scale; £3,333 to £5,627 p.a. (under review), depending on age and experience.

Further details and application forms from the Academic Registrar, University of Warwick, Coventry CV4 7AL, quoting Ref. No. 24/R/78.

Closing date for receipt of applications February 13, 1978. 1098(E)

QUEEN MARY COLLEGE

University of London

CHEMISTRY DEPARTMENT

Applications are invited for a Postdoctoral

RESEARCH FELLOWSHIP

in Organic Chemistry to investigate the porphyrin-N-oxides in collaboration with Professor R. Bonnett. The post would suit candidates well grounded in heterocyclic chemistry, but with an interest in biochemistry. The post is tenable for two years from July 1, 1978 (or as soon as possible thereafter). Initial salary in range (under review) £3,805 to £4,019 p.a. (including London Allowance).

Please apply by letter, enclosing curriculum vitae and names and addresses of 2 referees, to The Registrar, (N) Queen Mary College, Mile End Road, London E1 4NS. 1036(E)

ONTARIO CANCER INSTITUTE POSTDOCTORAL FELLOWSHIP

available to join a group working on the genetics and molecular biology of murine oncornaviruses and erythropoiesis. Candidates should be about to graduate in virology, biochemistry or cell biology in the spring or summer of 1978. Applicants should send curriculum vitae with three letters of references to Drs T. W. Mak or A. Bernstein, Division of Biological Research, Ontario Cancer Institute, 500 Sherbourne St., Toronto, Ontario, Canada M4X 1K9. Stipend will be \$11,600 to \$12,700 annually. 1093(E)

AUSTRALIAN NATIONAL UNIVERSITY

Applications are invited for appointment to the following:

RESEARCH SCHOOL OF BIOLOGICAL SCIENCES DEPARTMENT OF DEVELOPMENTAL BIOLOGY RESEARCH FELLOW

A Research Fellowship is available for two years, with possible extension, in preparative protein biochemistry to investigate the plant photoreceptor, phytochrome. Research is in progress to separate this protein in pure, native form. The appointee would take up this work and continue with the characterisation of phytochrome and with immunological studies on it.

CLOSING DATE: FEBRUARY 28, 1978.

DEPARTMENT OF NEUROBIOLOGY RESEARCH FELLOW

The position of Research Fellow is available in the Department of Neurobiology. The major requirement is that the applicant has the ability to sustain imaginative research programs in critical areas of invertebrate neurobiology.

The Department's present interests cover arthropod vision and hearing, but it wishes to diversify and any invertebrate neurobiologist with a Ph.D. degree is invited to apply.

Appointment for three years in the first instance with possibility of extension to five years.

CLOSING DATE: FEBRUARY 28, 1978.

SALARIES: Salary on appointment to the posts will be in accordance with qualifications and experience within the range: Research Fellow \$A14,851 to \$A19,426 p.a. Current exchange rates are \$A1 : 60p : \$US1.13.

OTHER CONDITIONS: Superannuation benefits are available. Assistance with housing is provided for an appointee from outside Canberra.

The University reserves the right not to make an appointment or to make an appointment by invitation at any time.

Prospective applicants should obtain further particulars from the Association of Commonwealth Universities (Apspts), 3 Gordon Square, London WC1H 0PF. 1039(E)

UNIVERSITY OF MELBOURNE POSTDOCTORAL RESEARCH FELLOWSHIP

in the

DEPARTMENT OF
MICROBIOLOGY

Applications are invited from Ph.D. graduates for the above-mentioned position which is funded by the Australian National Health and Medical Research Council and is available from January 1978 or soon after. The candidate will participate in a programme to develop continuous cell lines derived from immune lymphocytes. Previous experience in cytology, especially in the use of fusion hybrid techniques, would be an advantage. The position is available for two years with the possibility of renewal.

Salary: \$A17,279 per annum.

Enquiries will be welcomed by Dr W. Boyle, Department of Microbiology, University of Melbourne, Parkville, Victoria 3052, Australia. Applications referring to position number 526 355 should be addressed to The Registrar, University of Melbourne, Parkville, Victoria 3052. 1078(E)



MENTAL HEALTH FOUNDATION

8 Wimpole Street . London W1M 8HY

Telephone: 01-580 0145

Announces that

the Sir Geoffrey Vickers Lecture entitled

THE STUDY OF RELATIONSHIPS— IN QUEST OF A SCIENCE

will be given by

Professor Robert Hinde

M.R.C. Unit on the Development and Integration of Behaviour

Cambridge

at the

Middlesex Hospital Medical School, Cleveland Street, London W1 on Wednesday, February 8, 1978 at 5.30 p.m.

The Chair will be taken by

MR DUNCAN DEWDNEY, C.B.E., B.Sc.,
Chairman, Research Committee of the Foundation

All practitioners and students of medicine and its basic sciences are cordially invited to attend. Please apply for an invitation card to the

Secretary (N),

Research Committee,

Mental Health Foundation.

(There are no parking facilities)

1106(K)

UNIVERSITY COLLEGE GALWAY, IRELAND

DEPARTMENT OF PHYSICS

Applications are invited for a

POSTDOCTORAL

FELLOWSHIP

or

RESEARCH

ASSISTANTSHIP

for work on a project involving an investigation of the role of lead (and possibly other pollutants) in motor vehicle exhaust in the nucleation of ice in the atmosphere.

Support for the post which is tenable until the end of 1978 will come from the National Science Council.

Salary will be payable at the following rates:

Qualification	rate
Doctorate	£3,483 to £4,063 p.a.
Masters Degree	£2,835 to £3,282 p.a.
Primary Degree	£2,431 to £2,847 p.a.

Applications, including a full curriculum vitae and the names of two referees should be sent as soon as possible to

Dr A. F. Roddy,

Department of Physics

University College, Galway
from whom further details are available. 1042(E)

RESEARCH FELLOWSHIP

Postdoctoral Fellowship in Human Cell Biology tenable for two years in the first instance with possible extension for a third year, available from January 1978 for studies on the regulation of DNA, RNA and protein synthesis and on the expression of breast specific function in primary cultures of human normal and dysplastic breast tissue. Candidates should be experienced in cell culture and in the biochemistry of growth regulation.

Salary with entry according to qualifications and experience within range £4,649 to £5,669.

Further information from Dr R. Hallows (tel. 01-242 0200 ext. 307). Applications with curriculum vitae and names of two referees, should be sent to The Secretary, Imperial Cancer Research Fund, Lincoln's Inn Fields, London WC2A 3PX by January 31, 1978 (Quoting ref. 33/78). 984(E)

UNIVERSITY OF SURREY

DEPARTMENT OF

BIOCHEMISTRY

M.Sc./Ph.D. IN

TOXICOLOGY

Applications are invited from graduates in medical, veterinary, biochemical or biological sciences for places on a twelve-month MSc course in Toxicology for the session 1978-79. Applicants with some relevant experience in toxicology will be favoured. After completion of the M.Sc. degree, suitably qualified students may undertake a toxicological research study leading to Ph.D. A limited number of M.R.C.-supported studentships for the M.Sc./Ph.D. are available, but these are restricted to British subjects with a good honours degree.

The course is unique in providing a multidisciplinary postgraduate training programme in Toxicology which is run by the Department of Biochemistry with the participation and collaboration of the Medical Research Council Toxicology Unit, the British Industrial Biological Research Association and the Toxicology Laboratory of Shell Research Ltd. Additional contributions from, and visits to, other major academic and industrial research organisations also take place. The aim of the course is to give graduates an understanding and appreciation of the many disciplines involved in Toxicology, with particular emphasis on the molecular mechanisms of chemically-induced toxicity, and the assessment of toxicological hazards in the manufacture and use of new chemicals, pesticides and drugs. The course provides a broad biological education and is suitable preliminary training for industrial laboratories and Government agencies concerned with the safety assessment of chemicals, and research institutes concerned with the molecular interactions of chemicals with biological systems.


The closing date for applications is February 27, 1978. Forms and any additional information may be obtained from:—

Dr K. Snell, Department of Biochemistry, University of Surrey, Guildford, Surrey GU2 5XH (Tel. Guildford 71281, extension 822). 1091(D)

UNIVERSITY COLLEGE GALWAY, IRELAND RESEARCH FELLOWSHIPS ON HEAT PUMPS

Applications, stating qualifications, age and experience, are invited from Physicists or Engineers with experience to about postdoctoral level. Closing date is January 30, 1978.

Further information may be obtained from the Secretary, Department of Physics, University College, Galway (IRELAND). 1043(E)

Commonwealth  of Australia

QUEEN ELIZABETH II FELLOWSHIPS

Physical & Biological Sciences

To commemorate the Royal Visit to Australia in 1963 the Australian Government established the Queen Elizabeth II Fellowships Scheme. Under this scheme up to ten fellowships may be awarded each year for full-time research by young scientists of exceptional promise and proven capacity for original work. These are post-doctoral awards tenable in an Australian university or approved research institution normally for two years. Tenure of a Fellowship is expected to commence within nine months of the announcement of the award.

QUALIFICATIONS: Queen Elizabeth II Fellows must be either Australian or United Kingdom citizens. They should have a Ph.D., or equivalent

qualification, in one of the physical or biological sciences (which are deemed to include mathematics and the scientific aspects of statistics, engineering, metallurgy, agriculture and medicine). Awards will, in general be restricted to applicants who are not more than 30 years of age on the date when applications close.

STIPEND: \$15,888 (Australian) per annum – increased to \$17,077 per annum at age of 28 years.

ALLOWANCES: are payable in respect of a Fellow's dependent spouse (\$500 p.a.) each dependent child (\$200), superannuation payments (up to 10 per cent of stipend), appropriate insurance coverage and necessary travel expenses.

APPLICATIONS: Persons interested in applying for the above fellowships should obtain application forms and a statement of the conditions of award from the Secretary, Queen Elizabeth Fellowships Committee, Department of Science, P.O. Box 449, Woden A.C.T. 2606, Australia; the Minister (Scientific), Canberra House, 10-16 Maltravers

Street, London, WC2R 3EH U.K.; or the Consul General, Australian Consulate General, 636 Fifth Avenue, New York 20, N.Y., 10020 U.S.A. Applications for the next round of awards, which will be announced in June 1978 close at the Canberra address on 10 March 1978.

1054(E)



MENTAL HEALTH FOUNDATION RESEARCH GRANTS

The Research Committee of the Foundation exists to foster and support research in all fields bearing on mental health, mental illness and disorders of mental development.

The Committee meets twice a year to award RESEARCH GRANTS TO PROVIDE SCIENTIFIC ASSISTANCE, EXPENSES or EQUIPMENT for research workers.

For Grant application forms and further details apply in writing to:

The Honorary Secretary (N),
The Research Committee,
Mental Health Foundation,
8 Wimpole Street,
London W1M 8HY.

1104(H)

FRAME SYMPOSIUM

THE USE OF ALTERNATIVES IN THE
DISCOVERY, DEVELOPMENT & TESTING
OF THERAPEUTIC PRODUCTS
AT THE ROYAL SOCIETY
ON APRIL 11 & 12, 1978

April 11. The discovery and development of drugs—

Dr D. G. Davey, Chairman.

April 12. The testing of therapeutic products—

Dr G. E. Paget, Chairman

For further details contact Dr Andrew Rowan, FRAME,
312a Worpole Road, London SW20 8QU. Telephone 01-946 1450.

1084(M)



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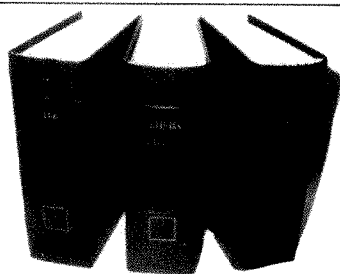
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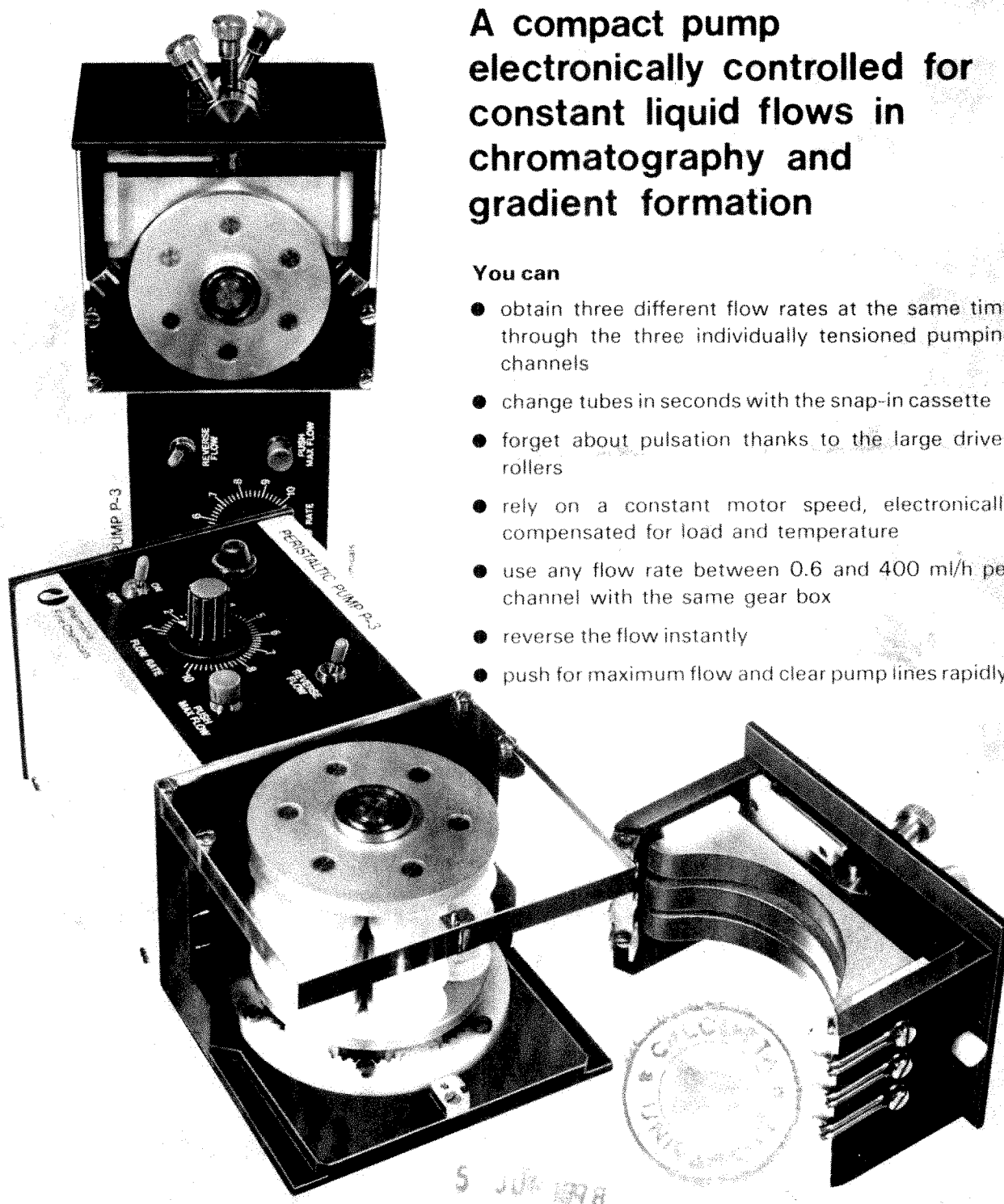
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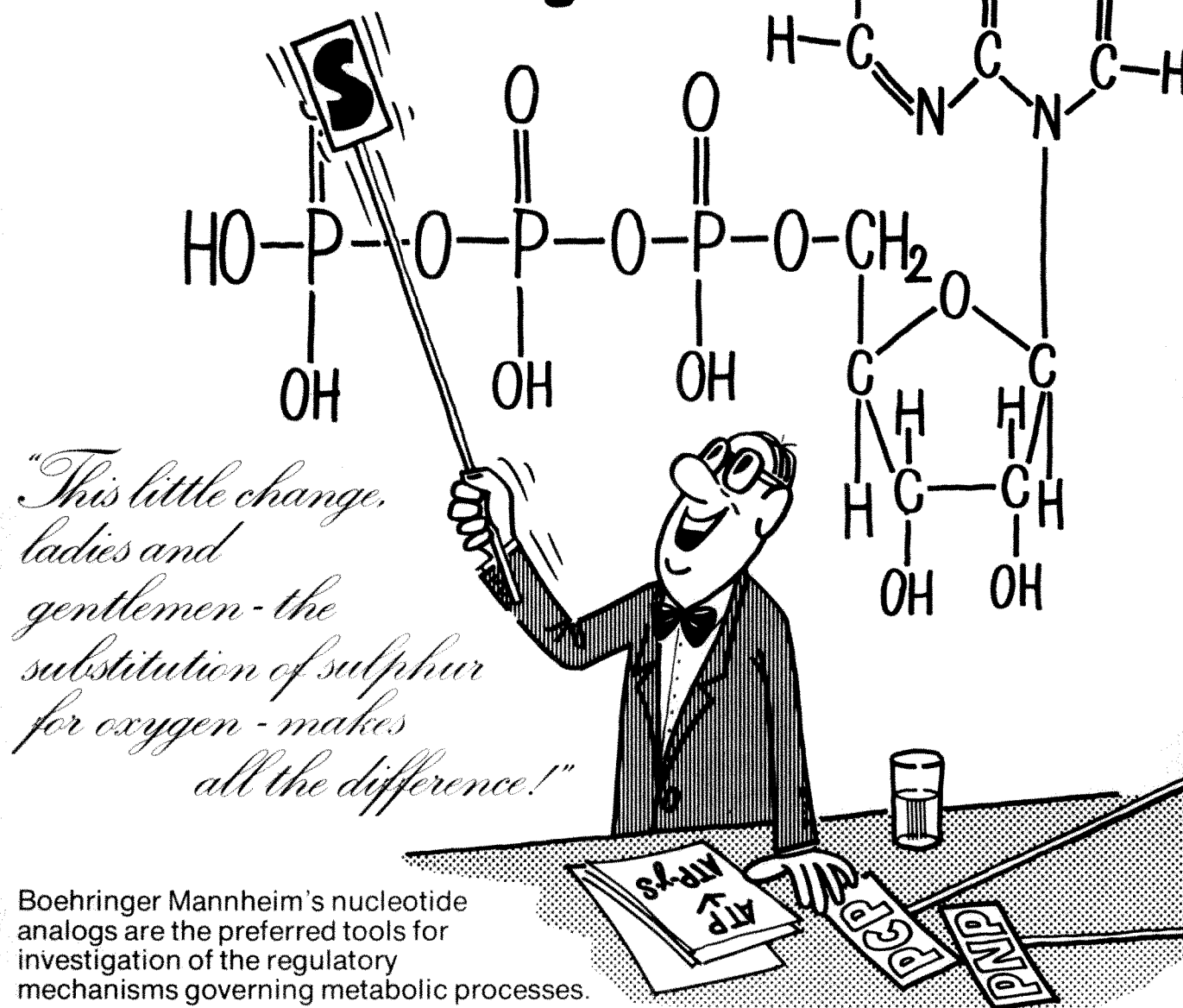
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Published weekly
ISSN 0028-0836

Registered as a newspaper at the
British Post Office

London
4 Little Essex Street, WC2R 3LF
Telephone: (01) 836 6633 Telex: 262024
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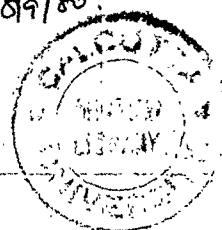
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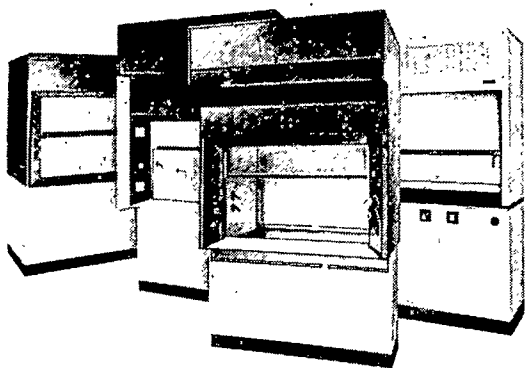
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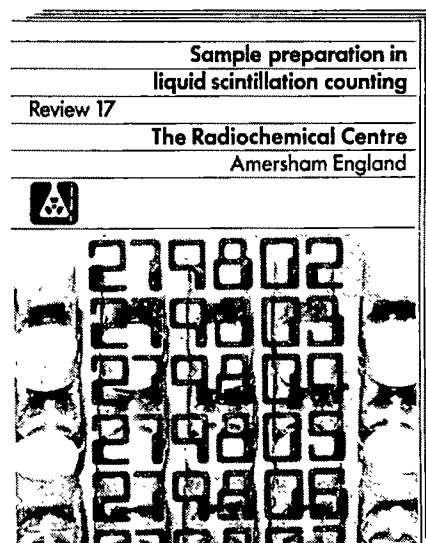
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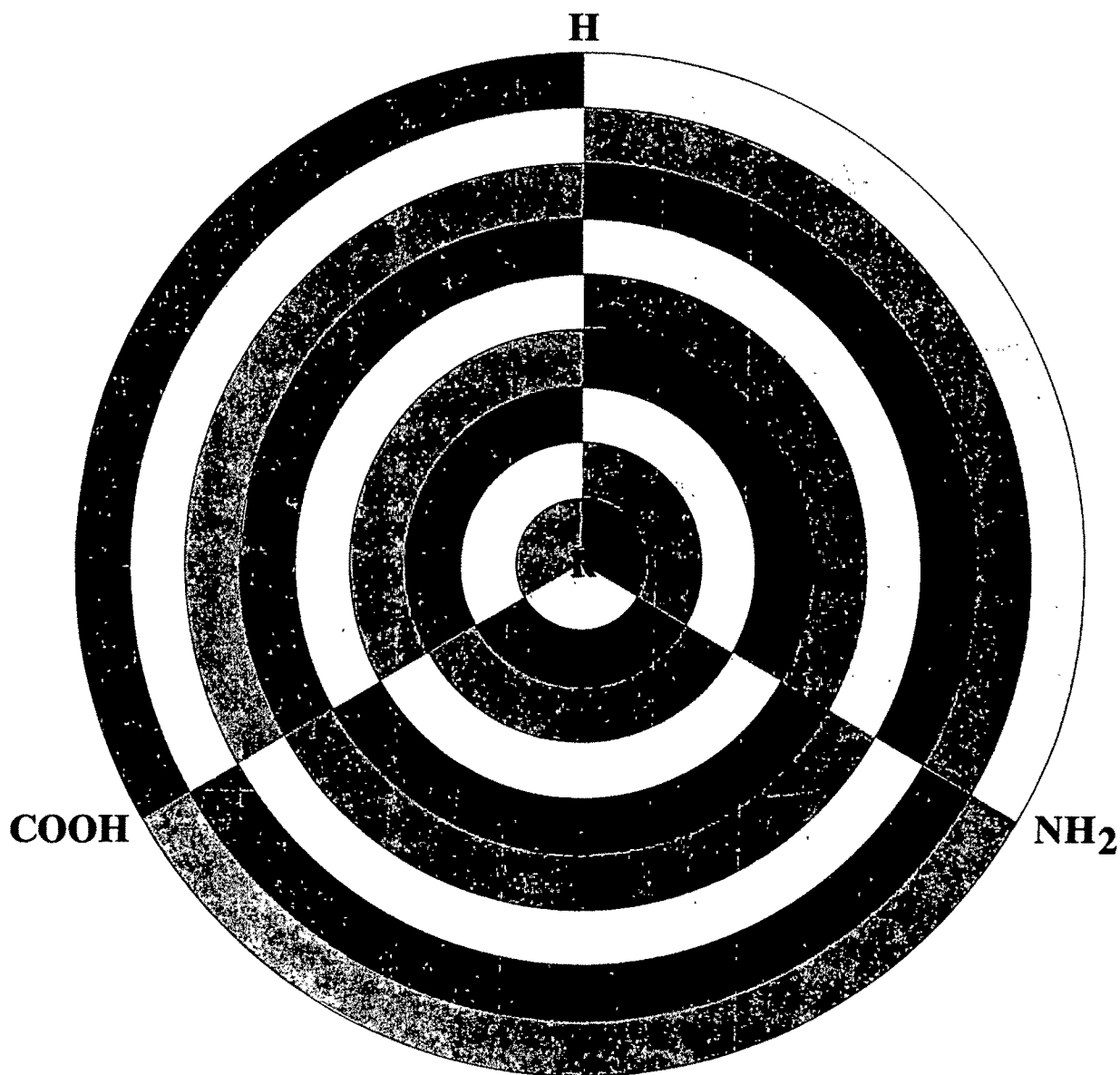
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26 January 1978

Science in the EEC still a problem

If there is one thing on which all involved European scientists and administrators agree, it is that a research and development policy for the European Economic Community is proving a mighty difficult thing to bring forth. There are many reasons for this. One is that there have long been doubts that the community of nine nations should be concerned other than peripherally in basic research—institutions such as the European Science Foundation, the European Molecular Biology Laboratory and CERN draw from a wider range of nations. Another is that applied science and technology policy is difficult to devise in the absence of a broad community-wide economic policy and the sort of political union which makes British taxpayers less agitated if the R & D funds they provide lead to the establishment of new industry in, say, Italy. Finally, the policy must take note of already existing community laboratories at Geel, Petten, Ispra and Karlsruhe—the so-called Joint Research Centre—a hangover from Euratom days when there was a naive and erroneous belief that the then community members, already lavishly equipped with their own nuclear research laboratories, would have work for yet another lavishly-equipped and wide open facility.

Add to this differences in the aspirations of larger and smaller nations and the tiny dimensions of the community's R & D budget in comparison with those of individual nations (1½% of the combined non-defence R & D budget of member states), and you might reasonably wonder whether it was worth the community persisting in trying to keep a scientific programme going. In July 1977, however, the Commission of the Community had a go at bringing some new order into the science and technology programme, with a document devoted to intentions for 1977-80 and draft resolutions and decisions embodying these intentions (*Nature* 268, 96). The House of Lords Select Committee on the European Communities has been considering the evolving policy and hearing evidence from scientists with European interests. Their report is now published (Commons Paper 37; £2.60). For those who imagine that any committee of Lords could hardly get to grips with such an esoteric matter, it should be added that the committee comprised several scientists and engineers, including Lords Ashby, Hinton and Zuckerman.

It was perhaps unfortunate that the committee had completed its gathering of evidence before the Commission's report was published, for although there were some interesting presentations on the European dimension to science and a particularly spirited contribution from Professor Pierre Aigrain, it is difficult to see some of the committee's conclusions on the community document emerging very clearly from their earlier deliberations; indeed one draft decision on industrial research which the committee commends does not seem to have been the subject of any discussion whatsoever. Even so, it is possible to discern from the general drift of the evidence that the community is of most value when it is used simply to co-ordinate national research ('concerted action'), some value in financial support of domestic projects of community-wide interest ('in-

direct action') and at its most problematical when the research is done in the Joint Research Centre ('direct action'). Not least of the problems in the last case is the bureaucratic constraint imposed on the actions of the director at Ispra which gives him less freedom than a laboratory director might reasonably expect. The freedom of Sir John Kendrew at EMBL to run his own show was not lost on the committee.

What, then, to do about the JRC? The community's document recommends, somewhat bravely, a mixed diet of nuclear safety, new energy sources, environment, resources and services. The Lords' committee suggest simply a "useful role in carrying out work not done elsewhere and, for example, 'ungrateful' research"—the latter being long-term unspectacular research. This, if anything, seems a recipe for even more dissatisfaction; a huge research laboratory devoted to unwanted projects is hardly likely to find a director able to give it even nominal cohesion or to prevent it from slipping rapidly into oblivion.

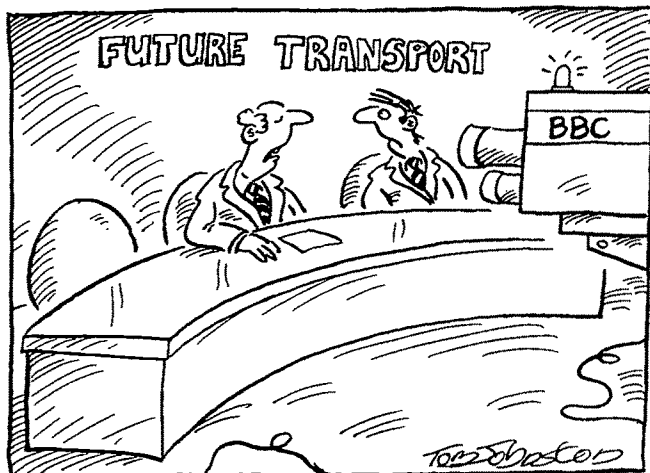
It is, however, the committee's views on forecasting which leave most to be desired. Some years ago the community established a group called Europe+30 to look into whether there should be long-term forecasting capacity, together with an Office of Technology Assessment, in the EEC. The group, under Lord Kennet, made its report in 1975 (now published in edited form as *The Futures of Europe* by C.U.P.). Its recommendations included the establishment of a permanent unit of between 30 and 70 people looking at all issues of concern to the community. Lord Kennet had a tough time when he appeared before the committee, which on that day notably lacked the scientists mentioned earlier. He or his team were accused of misuse of the English language, superficiality, amateurism, exceeding their brief and so on. But after the committee had heard from Lord Kennet and before the report was written, the community made its own move on forecasting. It proposed an attenuated version of Europe+30 called FAST—a forecasting-and-assessment-in-science-and-technology programme. This, as its name implies, would be restricted to one sector and would accordingly have a much smaller staff, probably of ten.

The Lords' committee, noticeably sceptical towards Europe+30, claims that FAST will be too diluted to be of value to the community. This is not argued out at all, and indeed could conceivably have gone the other way—that work in one sector would be more concentrated and hence of more value to the community. What is suggested in place of FAST is a scientific adviser, or team, reporting direct to the President. This is difficult to take seriously, particularly coming from Britain where, for better or worse, a scientific adviser reporting direct to the Prime Minister no longer exists.

Whatever happens in Brussels, it is time now for the Council of Ministers to take firm action. As the Lords remark, the ultimate responsibility for better organisation rests with ministers, and they had best wade into this problem with some vigour. □

Forecasting future transport

J. I. Gershuny, of the Science Policy Research Unit, University of Sussex, discusses the recommendations of the Department of Transport report of the Advisory Committee on Trunk Road Assessment (HMSO, January 1978)



"We were due to have a spokesman from TRRL here tonight . . . but he's been held up in the traffic!"

OFFICIAL procedure in the UK for assessing trunk road construction schemes has, in recent years, been the subject of increasing criticism. Statisticians and transport experts have claimed that the procedures are technically faulty. Local objectors to road building schemes complain that the obscurity of the methods prevents their participation in the planning process. The Leitch Committee, which reported recently, supported the technical criticisms, but did not suggest much practical help for protesters.

Decisions to build trunk roads in the UK are based on two technical procedures. One is a forecasting exercise carried out by a group at the Transport and Road Research Laboratory (TRRL) in Buckinghamshire. The other is a computerised cost benefit analysis package called COBA, developed by the UK Department of Transport and its predecessors. Both have come under increasing criticism.

The TRRL forecasting procedure is described in the report LR650 published by the laboratory. This document makes forecasts of vehicle numbers extending into the quite distant future—the final time horizon is the year 2010. It puts forward three alternative estimates of the car population of the UK at this date—25.0, 25.9 and 26.2 million cars, or 0.43, 0.44 and 0.45 cars per head of population respectively (LR650, p15). These alternative estimates are very close together by the normal standards of social forecasting; can we really be this certain?

The reason for the small range of the alternative forecasts is to be found in the forecasting procedure adopted by the TRRL group. The predictions are based on the assumption that the pattern of growth is logistic (S-shaped) over time. A logistic growth model tends towards a given saturation level at a rate determined by some causal variable or variables—in the TRRL case, national income, motoring costs and time. So the high estimate of 26.2 million cars in 2010 involves an assumption of high national income growth and low growth in motoring costs, the low 25.0 estimate assumes low income and high costs growth, and the middle one involves medium costs and growth.

A first reason that these estimates should bunch so closely together is that the very steady historical pattern of growth of vehicle numbers in the fifteen years preceding the fore-

cast showed little fluctuation with variations in incomes and costs. So when calibrating the model, the future effect of variations of prices and incomes is similarly assumed to be small in relation to the effect of the passage of time. Even large assumed fluctuations make little difference to the forecast—because on past evidence the price and income elasticities of demand for private cars is low. Sceptics doubt that historically derived elasticities necessarily hold for the future.

A second reason for the bunching is that built into the structure of the model is a tendency for the effect of the variables on the forecast to be progressively suppressed over time as the value of the saturation asymptote is approached. The saturation level assumed in LR650 is 0.45 cars per head, and since the final predictions are so very close to this we might reasonably assume that the causal variables are contributing very little to the final result. Finally, the saturation level itself is very much a matter of speculation. In spite of sophisticated presentation, the results of the exercise come down to little more than guesses.

The cost-benefit assessment package, COBA, is rather more straight-forward, being, of course, subject to all the standard criticisms of cost-benefit analysis. The system requires that planners put money values on monuments and artifacts that have no natural economic worth, but nevertheless have considerable cultural value. Furthermore, cost-benefit analyses inevitably leave some costs completely unconsidered; COBA itself takes virtually no account of environmental damage caused by road building schemes. And even were all conceivable impacts taken into account, the effect of using a market-based system of valuation is that the preferences of the rich are given much more weight than those of the poor in the assessment.

Further COBA's assessment of future benefits is based in part on the predictions of future car numbers—giving rise to the suspicion that the TRRL vehicle forecasts may be self-fulfilling. However arbitrary the original predictions are, if, on the basis of these forecasts, new roads are built, and if, as many people suspect, the number of cars increases to fill the available road space, then the volume of traffic may well grow to the forecast level—whatever that level is! Hardly a rational way of formulating policy.

It was this sort of criticism that led to the appointment of the committee of enquiry into trunk road assessment under the chairmanship of Sir George Leitch. The committee's report contains an extremely thorough and lucid review of the assessment methods as currently practised. It examines vehicle forecasting procedures and methods of economic and environmental evaluation and compares them with practices in France, the USA and West Germany.

The Leitch report recommends that the current vehicle forecasting methods should be scrapped. To replace them the committee suggests a modelling procedure that gives much more weight to those causal factors—such as household organisation, geographical location of facilities and the availability of public transport—which actually determine people's desires to own and use cars. Such models would in particular be sensitive to the effects of public policies altering the balance of costs and convenience between public and private transport; some observers suggest that encouraging the use of public transport relative to private might in the long term have an important effect on the growth of car numbers, but the present forecasting procedure is quite insensitive to such changes in policy. This sort of conditional information about the effects on vehicle use of alternative feasible public policies would be much more useful to policymakers than the present unconditional forecasts. The report—as far as it goes—is to be welcomed. □

Killer satellites

Farooq Hussain traces the history of the Soviet and US development of killer satellites

MILITARY operations depend heavily on satellites for intelligence gathering, navigation, weather information, command, control and communication. So their vulnerability to interference or destruction is a serious and legitimate concern of military planners. Satellites are delicate devices. To make one ineffective, it is necessary only to impair the performance of its sensitive equipment: its total destruction is unnecessary. As to means, radiation directed against a satellite can cause damage at much longer ranges than the effects of blast and flying debris from an explosion in the near-vacuum of space where shock waves have little effect. Nuclear warheads would be very effective against satellites—because of their lethal radiation. But the effects of nuclear anti-satellite weapons would be indiscriminate and would more than likely result in the destruction of friendly satellites as well as enemy ones. Killer satellites exploit the poor resistance of satellite components—particularly solar cells—to intense heating and radiation damage. High energy lasers can be easily directed at satellites, so their imagined use in killer satellites is extensive. Another possibility arises from the tendency satellites have to build up high electrostatic charges due to the higher electron density around the dark side of the planet. The use of ion beams directed at target satellites can cause arcing and discharge through the instrumentation either destroying or seriously damaging it. Precision guided missiles also offer the possibility of destroying satellites either by collision or by the use of a conventional warhead detonated close to the target.

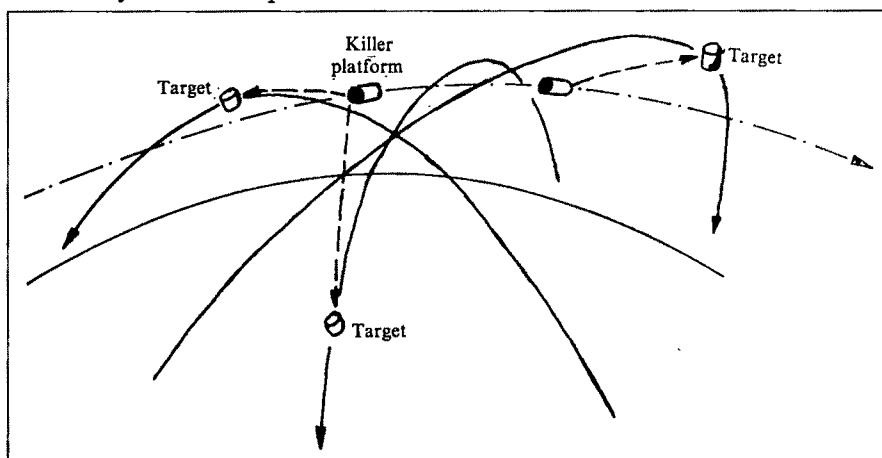
The United States and the Soviet Union have signed separate treaties in 1963 and 1967 first prohibiting testing and then deployment of nuclear weapons in space. But since 1968 the Soviet Union has been conducting tests within the general Cosmos series of satellites and these have been widely interpreted by western observers as an anti-satellite development programme. In these tests an orbiting interceptor is manoeuvred so as to make one or more close passes by a target satellite. Out of the 27 such Cosmos launches that have taken place to date only seven have ended with the explosion of the interceptor and these explosions have not always been in the general vicinity of the target satellite. The latest test in which the interceptor exploded in orbit

occurred in December 1977. Other tests have taken place which have suggested that new manoeuvring techniques have been adopted for the interceptor satellites.

American concern over this satellite programme has arisen because of the sudden resumption of testing after a lapse of four years, and the accelerated rate at which testing has been taking place. One explanation for the Soviet tests is the development of reconnaissance satellites by China. Soviet testing of the Cosmos series appears to have followed Chinese reconnaissance satellite development since 1970. In addition the orbits followed by the Cosmos series seem more appropriate to interception of Chinese satellites than American ones.

American test

The only American test of an anti-satellite system took place in 1963



The American system being developed by the Vought Corporation of Dallas, Texas

when a modified McDonnell Douglas Thor missile was fired against a missile booster in low earth orbit as the target. The Thor missile was brought within the range of the target for a simulated nuclear kill but the programme was dropped following the treaties banning nuclear weapons testing and weapons deployment in space. In a follow-on project the Vought Corporation built four infra-red homing guided missiles for use with conventional warheads. These missiles were to have been launched against satellites by a booster in a direct ascent trajectory towards the target without completing an orbit of the earth. Since the missiles were to have been armed with conventional warheads they needed a much smaller kill radius than for nuclear warheads and hence greater accuracy in guidance. But the first test launch suffered a booster failure and the second a

mechanical malfunction in deploying the payload. The programme was finally abandoned because of anticipated difficulties in getting the interceptor close enough to the target for conventional warheads to be effective.

Recent advances in the available energy, frequency range, and compactness of military lasers have prompted ideas that killer satellites may be able to carry high energy continuous wave or pulsed lasers to destroy target satellites. Ground-based lasers have been in use for a few years to interrogate reconnaissance satellites. The laser beam is used to illuminate the satellite, and the spectral pattern of the reflected laser radiation can be used to interpret various characteristics of the target. Using this technique it has been possible to determine the kind of equipment on the satellite and its sensitivity. Rumours that the Soviet use of lasers in this manner had tem-

porarily blinded an American reconnaissance satellite last year were persistent in spite of official US Department of Defense denials. However general concern within the US Department of Defense and Congress has resulted in substantial increases in the American space defence budget. \$61 million was authorised for FY 1977 with \$126 million requested for FY 1978 and an estimated \$265 million being requested for FY 1979.

In September the United States Air Force announced the award of a \$58.7 million contract to the Vought Corporation of Dallas, Texas for the development of a killer satellite. The project developed out of a competition begun in 1975 between General Dynamics/Pomona and Vought to produce an unarmed miniature homing missile designed to destroy satellites by force of impact on collision. The

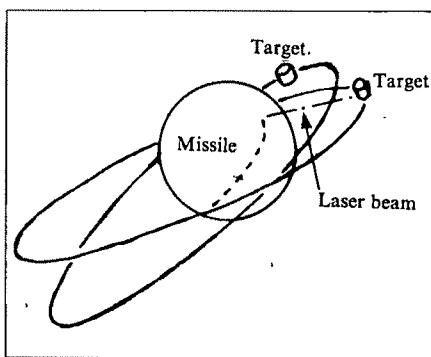
missile, about twenty centimetres in diameter and thirty centimetres long, is guided either by active radar or by far-infra-red seekers. Infra-red has special advantages because satellites give distinct thermal 'fingerprints'. More than ten homing missiles are carried into orbit by a highly manoeuvrable platform whose orbit is directed so as to provide the opportunity to fire missiles at the maximum number of target satellites within a minimum number of orbits. Free-fall tests of the infra-red guidance system have demonstrated mid-air activation, target acquisition and guidance to impact with a target. Electronic counter-measures against infra-red seekers (which are passive detectors, emitting no signal of their own) are more difficult than for active radar; and it is difficult for satellites to make rapid evasive manoeuvres in orbit. A similar system involving missiles armed with conventional warheads has also been under investigation.

Interpreting Soviet tests

By comparison, Soviet tests have only obliquely suggested a preference for destruction by large explosions in the vicinity of the target satellite. Since Soviet tests have never resulted in the physical destruction of a target, the explosion of the interceptor could be explained as a test of the vulnerability of the target to attack—rather than demonstrating the potential of the interceptor to destroy target satellites. The difficulty in interpreting properly the explosions in the Cosmos series also lends support to the idea that Soviet killer satellites may soon carry laser or ion-beam weapons. In any case the Soviet and American hunter-killer satellite developments should not be directly compared because each Super Power has very different approaches to the development of major weapon systems.

Presently US space defence research is concentrated upon problems of hardening satellites against attack and increasing the resistance of communication, command, and control transmissions to jamming. These battlefield transmissions now rely almost

completely on satellites. The third generation of American military communications satellites now being developed employ a technique of using multiple directional beams for transmission, and of shifting frequencies in the ultra and super high frequency bands (7 to 8 GHz) to avoid the effects of jamming. Satellite antennae are being developed that use electronic counter-measures to eliminate the effect of a directional jamming beam fixed on the satellite. Solar cells are being designed and built to withstand the effects of intense heating and radiation, by using filter coatings which do not absorb energy at typical laser wavelengths, and by using construction techniques with materials with high resistance to heat and radiation damage. The vulnerability of solar cells has led to an increased interest in nuclear power sources for satellites which until now have always



The Soviet system can only knock out one satellite at a time making it ineffective against the US global positioning array of 36 satellites

had the disadvantage of being much heavier and considerably more costly to operate. Three designs are currently under consideration all of which use P^{238} isotopes as the power source. With the considerable improvements in performance that are being achieved for satellites military dependence on them can be expected to increase throughout the 1970s and early 1980s. During the first half of the 1970s military missions accounted for about half of the American satellite launches and both the number of military satellites and their relative size and payload will increase further as the NASA Space Shuttle

begins operation.

The development of killer satellites is unlikely to provide any significant strategic advantage for either Super Power and the race to develop them is likely to be very costly. A surprise attack is hard to achieve with killer satellites because the preliminary manoeuvres are easily detected by other satellites. It is also possible to place in distant orbits satellites which are ready to replace ones destroyed by an attack. These so-called 'dark' satellites have characteristics such as very small radar cross-sections and low reflectivity which make them very difficult to detect and track. Because of their distant orbits they are relatively safe from attack in the first instance. The real advantage of destroying military satellites in a strategic nuclear conflict would appear only if one side destroyed a significant number of the opponent's satellites while retaining its own. A rapid destruction of all satellites would yield no advantage to either side.

It is perhaps to avoid a costly arms race that both the Soviet Union and the United States are anxious to reach an agreement limiting the development of killer satellites. The United States was reported to have requested Soviet consideration of such an agreement last March. The Soviet response was favourable and the United States National Security Council are presently considering a formal policy based on reports by various departmental agencies on the implications and verification problems of a hunter-killer satellite limitation or ban. The intention is to provide for an agreement separate to SALT and talks on a possible agreement over limiting killer satellite development may begin this Spring. Meanwhile US Defense Department officials are concerned that present programmes should not be effected by the agreement and it is unlikely that the incentive given to US space defence research by the recent Soviet testing of the Cosmos satellites will be abated by a limitation—unless the form of the agreement is radically different to those which have been made in other areas of strategic arms control. □

1979 looks like a good year for US science

CLIMATOLOGY, reproductive and developmental biology, and military-related basic research are three areas of science likely to receive a major boost from President Carter's Budget for the fiscal year 1979, which was submitted to Congress on Monday.

Other areas to benefit from a budget that proposes an overall increase of 10.9%—about 5% above expected in-

flation levels—for basic science include solar studies, which is given the green light for two major projects, and earthquake research. Potential casualties include one of the five planned space shuttles, and the Clinch River liquid metal fast breeder reactor demonstration project. The proposed termination of the Clinch River project would save about \$150 million, and would

result in virtually no increase in the energy research and development budget overall.

In general, the budget carries an optimistic message for basic science, in line with the results of a broad-ranging survey of the nation's research activities carried out at the President's request by the Office of Science and Technology Policy (OSTP). The projected

increase in spending on basic research is precisely the level recommended by the President's Science Adviser, Dr Frank Press, who claims "100% support" from the Office of Management and Budget.

But if previous budgets could be described as Keynesian, this one returns to a more classical, monetarist mould. Emphasis in funding is shifted away from development projects (for example in solar heating technology) which are felt to be the responsibility of the private sector and its market forces, back to more basic "long term" considerations.

Thus within an overall increase in support for research and development of 6.1%, to a total of \$27,900 million, the main increase will take place in basic science, from \$3,288 million in 1978 to \$3,647 million in 1979. In contrast the budget for applied research will increase by only 7.4% from \$6,248 million to \$6,711 million, and for development by an even lower rate of 4.6%, from \$16,753 to \$17,532 million.

A major beneficiary of the increased funding for basic research will be universities and colleges, many of whose problems—such as the obsolescence of equipment and the lack of opportunities for young scientists—were brought to the President's attention by the OSTP survey. Total R & D support for colleges is therefore suggested to rise from \$3,300 million in 1978 to \$3,561 million in 1979, an overall increase of 7.9%, with particularly large increases in funds for agricultural (10.7%), energy (11.7%), and military research. (In the light of the benefits which these increases are expected to bring, the NSF's proposed basic research stability grants, whose \$4.5 million was "impounded" by the President from the 1978 budget a few months ago, will be permanently dropped.)

One area to receive a major boost is climate research. A broad initiative in this area, involving eight agencies and a 39% increase in funding to \$104 million, will be coordinated by the National Oceanic and Atmospheric Administration of the Department of Commerce.

Three important aspects of the proposed 1979 budget will be: the development of satellite sensors for ozone and earth radiation monitoring—a major field study of the equatorial Pacific—and basic research on carbon dioxide levels in the atmosphere and oceans.

Two new programmes are included in the budget of the National Aeronautics and Space Administration (NASA). The first of these is the Solar Polar Mission, a joint project with the European Space Agency to send two space craft, launched from the Space Shuttle in 1983, past Jupiter, in an orbit that will permit the first study of

the polar regions of the sun. And the second project is the Solar Mesospheric Explorer, which will permit study of the effects of solar radiation on the earth's ozone layer.

At the Department of Health, Education and Welfare, whose basic research budget is proposed to be increased by 14.9% to \$992 million, areas scheduled for increased support include reproduction and family planning, developmental biology, and child development, as well as the potential environmental hazards to human health.

There are bright prospects, too, for military-related basic research. Spending by the Department of Defense is proposed to increase by 14%, almost as much as for health research, reflecting the view of Defense Secretary Dr Harold Brown that this area has been underfunded in recent years.

One result of this will, according to Dr Press, be the re-establishment of connections between the military establishment and the university research community which, he says, "have been allowed to lapse in recent years" and the R & D budget of the Department of Energy includes provision for work on the controversial neutron bomb.

The future for basic research—at least in most areas—therefore looks better than it has for several years past. But the *leit-motif* remains that of the ultimate pay-off. Dr Press, pointing out that the applications of basic science may take 10 or 20 years to come through, characterises this as an area in which industry tends as a consequence to "underinvest", thus justifying increased federal expenditure. And Dr Robert Frosch, administrator of NASA, ended his statement on the Agency's budget with the words that it would "give the American taxpayer the return on his investment he expects and deserves".

This approach is also reflected in the budget of the NSF. One area due for significant expansion, for example, is the integrated basic research programme of the new Applied Science and Research Application Directorate.

This is scheduled for an increase from \$1.9 to \$7.0 million, and will be concerned with "identifying basic research relevant to significant national problems", jointly with the basic research directorate of NSF.

One area to have suffered from this cost-conscious approach is high energy physics, whose budget is planned to expand by only 6.7%. The budget allows substantial sums for the construction of the intersecting storage accelerator (Isabelle) at Brookhaven, and for a new high-intensity beam facility at the Lawrence Berkeley Laboratory.

At Fermilab in Illinois, however, although the energy saver/doubler project is being formally moved from the R & D to the construction phase, only \$10 million of the proposed authorisation of \$38.9 million for construction is scheduled to be spent in 1979—and this will be partially met by reducing the \$86.1 million authorised for running costs in 1978 and \$81.8 million authorised in 1979.

But if the cost/benefit consciousness that runs through the proposed budget has resulted in decisions that are unwelcome in some quarters, it is likely to increase the chances of the budget proposals being looked upon favourably by Congress.

Last year, the substantial increases for research and development requested by President Ford in his outgoing budget were received sceptically. Although making a considerable, and almost traditional, increase in the NIH budget, Congress reduced the request for the NSF, for example, from \$885 million—a figure which NSF Director Dr Richard Atkinson admits "we were not able to defend"—to \$863 million.

This year the OSTP has done the President's homework. And there will be both substantial evidence, and the carefully-considered support of departmental and agency heads, to back up demands in the face of Congressional cross-examination. So the signs are that 1979 will be a good year for scientists.

David Dickson

Federal funding for conduct of R and D by Agency (in millions of dollars)

Department or Agency	Obligations		% increase 1979 over 1978
	1978	1979	
Defense-military functions	11,709	12,740	8.8
Energy	4,231	4,245	9.3
NASA	3,877	4,193	8.2
HEW	3,137	3,258	3.9
NSF	754	829	10.0
Agriculture	626	632	1.0
Environmental Protection	351	358	2.0
Transportation	364	342	-6.0
Interior	366	340	-7.1
Commerce	288	316	9.7
All other*	587	637	8.5
TOTAL	26,287	27,800	6.1

*Includes the Department of Justice, Labour and State, The Veterans Administration, The Civil Service Commission, the Corps of Engineers, the Federal Trade Commission, the Tennessee Valley Authority, and the Library of Congress.

Anti-relativist draws others into the whirlpool



Stefan Marinov

*O father Galileo, cunning one and wise,
Thy trial persisteth still even from age to age;
Moralist and philosopher try thee, the fool eke tries,
And everyone who counts himself a learned sage.*

*So wast thou then a coward, valourless, without honour,
Thyself knowing the truth, to spit on truth, deride,
Saving thy mortal frame, to fraud to sing "hosanna",
Before all men to trample thine honour and thy pride.*

*Holy lord of the spirit, my teacher wise and dear,
Is the common herd worth our torments and our blood.
Shout yourself hoarse—no sound will reach its blunted ears;
Throw your heart at its feet—onward it still doth plod.*

*So, doctors, I bow and swear: "There is no absolute space!
All I affirmed is lunacy—bring on your drugs apace!"*

RESTRICTIONS on scientific correspondence in the Soviet Union and its satellites have, over recent years, become familiar—the classic study of the problem being *The Medvedev Papers*. One of the most curious by-products of the system is the recent appearance, in Belgium, of an anti-relativistic tract with the lofty title *Eppur si Muove* and a preface signed by no less a person than A. D. Sakharov—presumably the dissident academician of that name. In fact, as the author of the book, Stefan Marinov, himself admits, Sakharov never wrote such a preface; Marinov claims, however, that Sakharov gave him permission to append his name to a preface written by Marinov on his behalf.

Although this may appear at first glance somewhat a trivial matter—that of a 'fringe scientist' trying to gain the backing of an eminent member of the orthodox community—the appearance of the preface could have considerable implications for Academician Sakharov. The various campaigns launched against him within the Soviet Union regularly imply that he has 'abandoned' or 'betrayed' science for 'so-called dissidence', and his apparent endorsement of a scientific theory which he himself does not hold, simply because its author was himself in trouble with the authorities in his own country, could add valuable fuel to this debate. The history of this curious preface is therefore worth looking into.

Stefan Marinov first made his appearance in the western media in the autumn of 1976, when large advertisements began to appear for a conference on 'Space and Time Absoluteness' the following May, on his initiative. A certain 'A. D. Sakharov' of Moscow was listed, variously, as Chairman or Patron of the conference. This surprising announcement led to considerable speculation, and a general consensus of opinion that it could not be Academician Sakharov who was meant. Even the difference in spelling

was cited to support this idea, by those who did not realise that the Russian name САХАРОВ would, in certain transliteration systems, be rendered as 'Sacharov'. In fact at the time of the announcement, Marinov and his western supporters were still trying to contact Academician Sakharov by telephone, to ask for his consent, and were approaching anyone (the present author included) whom they felt might be able to make such a contact.

Marinov's next attempt to contact Sakharov came the following spring. His *magnum opus*, refuting the theory of relativity and all associated physics, was ready for publication, and he wished Sakharov to provide a preface. Having still failed to contact Sakharov over the Varna Conference, Marinov wrote the preface himself, distributed copies to possible contacts with the request that they forward them to Sakharov, and added a covering letter which, in the manner of a student applying for an *exeat*, said that unless he heard to the contrary, he would assume that he had Sakharov's permission to proceed. In one version of the covering letter, he added a brief self-portrait. "As far as I know, I am the unique 'dissident' in my country (once in a prison, twice in a loony bin). I descend from an old family of intellectual communists, and I am a Marxist (I have even written a book on mathematical political economy—in Russian—and I have a translation in Serbo-Croatian). My opinions are most close to those of Roy Medvedev."

Marinov was soon to be back in the mental hospital for a third time. At the end of April 1977, telegrams signed 'Marinov' were sent to journalists and others who had any connection with the Varna conference, cancelling it on the grounds that an earthquake was expected. The immediate assumption, that Marinov had taken this means of cancelling an event which had no supporters, proved false. Marinov had been removed to hospital by the authorities,

who had then notified in his name all those on his address list. News of this reached the West in May, but journalists were earnestly requested by his friends not to publish, since this would endanger his life. In all events, once the critical dates of the planned conference were over, Marinov was released, and in late summer he was allowed to emigrate. He settled in Belgium.

In October, 1977, the news-magazine *Pourquoi Pas?* carried a massive article on Marinov, 'The Scientist who came in from the cold', with a reprint of the 'Sakharov' preface. This, allowing for translation and editorial omission, was identical with Marinov's own draft. Although it seemed highly unlikely that Sakharov would have lent his name, I decided to seek confirmation on this point. It is virtually impossible to get a letter through to Sakharov, and direct telephone contact is likewise a random matter with minute probability of success. Nevertheless, the message reached Sakharov by two channels, and two answers were received. One, via a physicist, ran 'Academician Sakharov knows of the book, but did not wish to be associated with it, as he does not agree with the theory!' The other, less formal message, was transmitted as 'Andrei Dmitrievich says: "The man's a nut-case (*psikh*), but I wouldn't want to condemn anyone to a mental hospital!"'

At the end of November, Marinov turned up, uninvited, at the Science Session of the Venice Biennale. Asked about the preface, he maintained that a 'courier', described as 'an eminent physicist' and a 'young girl', had taken the book to Sakharov who received the courier, expressed sympathy for Marinov's plight, and agreed to 'think about' the matter of the preface. Sakharov is well known for his kindness and compassionate interest in all those in trouble; and he probably meant simply to give an expression of personal sympathy coupled with a polite refusal

to involve himself with Marinov's theories. Unfortunately, Marinov construed this as consent to have his signature added to the preface. Although a number of people entreated Marinov to withdraw it, he refused, saying that as it had appeared in *Pourquoi Pas?* it was now too late to do so. Moreover, he needed Sakharov's name to sell the book; unless he could sell 5,000 copies at \$20 each he could not get the money he needed to carry out the experiments described in it. (One presumes he meant 'replicate'.) A long and hysterical telex was dis-

patched to Sakharov c/o the Soviet Academy, and copies circulated among the Biennale journalists. Sakharov at that time was not even in Moscow; he and his wife were staging a sit-in in a Siberian labour camp where her nephew Edvard Kuznetsov, the dissident writer, had been refused his regular visit from the Sakharovs. At the time of writing, Marinov is still trying to get a message through to Sakharov.

Marinov's experiences in defence of his theories have undoubtedly made him only the more adamant in maintaining them. His poems imply that

his incarceration in the mental hospital was on account of his theories (see sonnet opposite). Clearly he is willing to take any means to promulgate them, even resorting to 'short cuts' when no answer is forthcoming. This is almost certainly not the first such occurrence in the long history of East European censorship—a number of very curious documents have reached the West from time to time. The whole episode is yet another illustration of the curious situations which can arise when governments restrict the freedom of scientific contact and correspondence.

Vera Rich

Polishing a tarnished image

LAST Wednesday the centre of Washington was brought to a standstill by a demonstration of angry farmers demanding "100% parity"—a price for their products that would give them the buying power of 65 years ago, when agricultural prices were at their peak. That same afternoon, a group of congressional employees was given a seminar on "government's role in scientific research" by a group of distinguished biomedical scientists, including three Nobel laureates—Arthur Kornberg, George Palade and James D. Watson—and the heads of some of America's leading biomedical research institutions and medical schools.

The style was different from that of the farmers, but the demand was very similar: a return to the levels of funding that basic research in the biological and medical sciences enjoyed in the relatively halcyon days of the late 1960s.

The case that the scientists presented during a well-organised two-day visit to Washington—which included private meetings with congressmen and members of the administration, as well as public hearings before the appropriations committees of both the Senate and the House—was straightforward. Basic research, they claimed, is grossly underfunded in comparison with applied research—it is in a chronic state of instability and lacks the means of training a new generation of scientists.

"We are here to draw to the attention of our legislators the importance of basic biological research in the solution of major elements of our nation's health care problems," Dr Mahlon Hoagland, President of the Worcester Foundation for Experimental Biology, and a major organiser of the Washington visit, told Senator Thomas Eagleton's appropriations subcommittee on the budget of the Department of Health, Education and Welfare.

On the surface, the argument was

about money; the report in the *New York Times* carried the not unfamiliar headline "Scientists plunge into lobbying for more medical research aid". And the scientists presented a carefully-quantified list of grievances.

For an example they claimed that there has been an 18% drop in the total amount of federal funds spent on basic research since 1967, and a reduction of 17% in support for scientist-initiated grants awarded by the National Institutes of Health (NIH) between 1967 and 1975.

Between 1967 and 1977 there was a decrease in the proportion of grants funded to grant applications submitted from 53% to 33%, they told the subcommittee. And the scientists also pointed out that there has been a decrease in funds for training young scientists from 18% of the NIH's extramural budget in 1967 to 6.8% in 1976.

The demands, too, were specific. The group said that it wanted the NIH to be provided with an across-the-board increase in funding of 10% in the fiscal year 1979 to compensate for the effects of inflation, and a return to 1967 levels in both scientist-initiated grants for basic research (then 61% of the NIH external budget) and the biomedical research support grants system (then 7%).

In addition, they requested an extra \$100 million a year for five years (an increase of almost 50% over the current budget) to be added to the budget of the National Institutes of General Medical Science, the NIH's basic research institute through which many biomedical research activities in universities and medical schools are funded.

Yet the visit to Washington was not only—or indeed primarily—about money. Indeed on purely statistical grounds, the case that the scientists presented lay open to criticism. It was pointed out, for example, that by taking the 1967 figure as a bench-mark, a

year in which research funding is generally reckoned to have reached the peak of the 1960s expansion, figures for subsequent years appear particularly—and perhaps artificially—bad. And figures presented purely as percentages obscure the almost 300% increase in total funding for NIH.

Furthermore both NIH and President Carter's Office of Science and Technology Policy have shown themselves to be aware of the current problems facing the basic research community. After what everyone agrees was a bleak period between 1967 and 1972, funds for basic research have been picking up, and will continue to do so if Congress accepts the suggested increases in President Carter's budget proposals presented this week.

But behind the dispute over financial resources lies a deeper issue of concern to the scientific community, the public image of science, and in particular of basic science on which Congress's willingness to provide additional funds ultimately stands.

In recent years, just as the debate over the implications of the Rothschild Report in Britain have reflected growing demands for the "relevance" of medical science, so similar tendencies in the US have given rise to what has been called the "disease of the month" mentality with a philosophy that medical science should be primarily directed towards curing, rather than understanding a disease.

In this climate, as funds have come pouring in for research into disease-related programmes such as cancer and heart disease, resulting in the total NIH budget increasing from about \$1,000 million to over \$2,500 million in seven years, so basic research has—in relative terms—lagged behind, and the process of scientific discovery has, it is claimed, been both distorted and delayed.

In the eyes of many basic scientists the villain of the piece is the so-called "war against cancer" launched in 1971

with President Nixon's much-heralded National Cancer Act, and responsible for an increase in cancer research funding from \$180 million in 1970 to almost \$900 million in 1978.

No one is claiming that the money has been entirely wasted. Much good science has been carried out under the cancer programme, and despite occasional widely-publicised lapses, few are prepared publicly to identify specific projects which they feel should not have been funded.

But various factors have led to a cooling off in Congress' initial enthusiasm for a massively-financed cancer research programme, and to increasing demands for a visible pay-off from its investment. These factors include data showing that, in spite of all the research and clinical advances, deaths from cancer continue to increase, and the growing evidence that many cancers are due to environmental causes, accessible to preventive rather than curative techniques.

Congress' frustration at the lack of tangible results reinforces the view of those scientists who criticised the whole "target-oriented approach", to biomedical research funding from the beginning. The fear, however, is that a failure of strategy could result in a general disillusionment with the whole research enterprise, affecting both its basic and applied aspects.

Dr Arthur Kornberg, for example, professor of biochemistry at Stanford University, claims that it is very rare for a crash programme of biomedical research to succeed in its objective, but warns that failure also carries its price. "Progress in medicine rests on fundamental advances. You harm people by trying to do things prematurely—you destroy your credibility, and the whole of science suffers. We have seen this happen in recent years, and people are discouraged from entering a field which has come to be regarded as bad science."

Dr Kornberg criticises the extent to which research workers are increasingly required to keep their eyes on a fixed target. "At present we have to boot-leg under various guises if we want to carry out fundamental research. This has both the spirit and the content of scientific investigation."

The task therefore facing the biomedical community, confronted by an apparent failure to come up with the goods that congress—many feel unjustly—has demanded, is how to make it respectable to be seen giving money to basic research in the biological and medical sciences. Criticism of the cancer programme, for example, will be to no purpose if the net result is an overall reduction in NIH research funds, rather than a redirection of



Dr Arthur Kornberg

funds towards basic research.

Furthermore Congress has no direct control over the way that the directors of the various NIH institutes distribute the funds allocated to them. A suggestion that each institute be required to spend at least 30% of its budget on basic research would be "a mistake" according to Dr Donald Frederickson, Director of NIH, who points out that most institutes do this already.

The best that scientists can hope for is some recommendation from Congress that it would like to see the various institutes upgrade basic research, a suggestion to be checked later against actual performance. "I feel that some of the institutes have tended to overemphasise target research at the expense of basic research because that was what Congress wanted. But Congress might now take the opportunity to communicate its belief in the importance of basic research," Dr Seymour Kety, Professor of Psychiatry at Harvard University and a past scientific director of the National Institute for Mental Health told the Senate Appropriations Subcommittee.

So far, the response of several key Congressmen has been relatively favourable. Sensing a growing disillusionment with the target-oriented approach to research funding, they are prepared to back the case that the long-term solutions lie in supporting basic research.

Mr Eagleton, for example, told the scientists that his subcommittee had "become aware of the pitfalls brought on by the proliferations of targeted research programmes." And Representative Paul D. Rogers, Chairman of the House Subcommittee on Health and the Environment, has promised to introduce a research training grants bill that would provide an extra \$220 million in 1979.

But the case is far from conceded. Cancer research still has powerful supporters who, while accepting that it is

the quality rather than the quantity of research that matters, claim that this means more rather than less money. And there remains the widespread feeling in many quarters—frequently voiced, for example, by Senator Edward Kennedy—that medical scientists should be required to produce results of visible social usefulness to justify the large investment of public funds that they receive.

Thus in spite of a significant increase in federal support for basic research in the proposed budget for 1979, basic biomedical science is unlikely to achieve its "100% parity" with the boom years of the late 1960s. At least not in the near future. The most that scientists can hope for is that they can encourage the pendulum to swing a little faster in their direction.

David Dickson

Lederberg named President of Rockefeller University

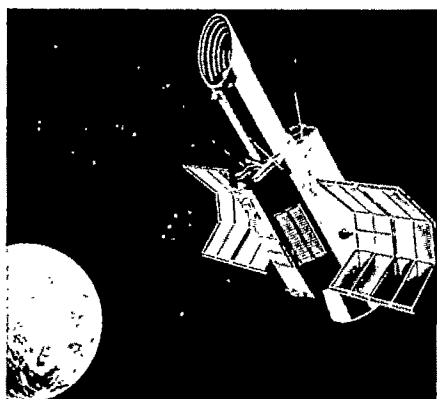
Professor Joshua Lederberg, Chairman of the Department of Genetics at the Stanford University School of Medicine, and Nobel prize winner in 1958 for his work on the organisation of genetic material in bacteria, has been elected President of the Rockefeller University in New York. Professor Lederberg will take up his appointment from 1 July and succeed Dr Frederick Seitz.

UK science budget announced for 1978/9

Spending on science through the UK research councils will be up by more than 2% in real terms in 1978/9, claim the Advisory Board for the Research Councils (ABRC) following agreement between the Board and the Secretary of State for Education and Science on expenditure levels. The Science Budget, which funds the research councils, Natural History Museum and Royal Society, is £256 million at 1977 prices; the Science Research Council get £139 million of it. The figures include two increases recently authorised, one for a recurrent £4 million following a slight easing of the economic situation, the second for a once-off £4.5 million to stimulate the construction industry.

The distribution of the budget still reflects ABRC's intention gradually to redeploy resources away from big science but there is now sufficient leeway with the extra money for one person at SRC headquarters to describe the mood there as "reasonable satisfaction, even modified rapture".

International Ultraviolet Explorer to be launched this week



DURING the course of evolution some animals have developed eyes on stalks as a rather bizarre adaptation to their environment. Astronomers could well do the same to escape the disturbing effects of the earth's atmosphere but evolution is a slow process and, luckily, there are more acceptable solutions. For many years now, astronomers working at wavelengths that are absorbed by the atmosphere have been sending probes into space, but the available techniques have not been entirely satisfactory. Rockets and balloons provide only fleeting glimpses and orbiting satellites not continually in communication with the ground must be automated to collect data blindly for later transmission. A solution is required which enables the astronomer continuously to control and monitor the performance of a telescope that remains above the atmosphere for long periods of time.

That solution is provided for the ultraviolet (UV) wavelength region by the International Ultraviolet Explorer (IUE) satellite scheduled for launch into geosynchronous orbit on 26 January. The satellite will be continuously visible from a control station on the ground and can be used just as if it were a ground-based UV telescope not suffering from the effects of the atmosphere.

In the ultraviolet region those effects are disastrous. The earth's atmosphere becomes increasingly opaque at wavelengths below 4,000 Å and is effectively 'black' beyond 3,000 Å. Balloons can observe over most of the mid-ultraviolet range from 3,000 Å to 2,000 Å by ascending above the bulk of the ozone layer to heights of 40 km, but for the far ultraviolet below 2,000 Å it is necessary to go much higher. The planned orbit of IUE varies from 25,000 km to 46,000 km altitude.

There are sound reasons for wanting information at ultraviolet wavelengths. The sun is brighter at visible wavelengths than in the far ultraviolet but this is not true for many other stars. As the temperature of a source

increases, the emitted radiation appears at shorter wavelengths and at 20,000 K, a typical surface temperature of a newly-formed star, almost all of the light is at wavelengths that cannot penetrate the earth's atmosphere.

The cooler regions between the stars are also important at UV wavelengths. Interstellar gas contains about 10% of the material in our galaxy and can be detected only by the characteristic absorption lines it imposes on the spectra of objects observed through it. The gas constituents are in their lowest energy state because of the low densities and temperatures in space and so it is necessary to observe at wavelengths where the atoms and molecules in their ground states can absorb light.

The common elements in interstellar gas are hydrogen, helium, oxygen, carbon, nitrogen and silicon. They all have their strongest absorption lines below 3,000 Å, with only the less abundant sodium and calcium appearing in the visible region. Therefore the major constituents of interstellar gas clouds can only be observed by measurements in the UV. If the gas clouds are moving, then the absorption lines will be Doppler shifted in wavelength and high resolution measurements of the spectra allow their velocity to be calculated.

Curiously, UV emission from some of the stranger objects in the sky has been studied from the ground. Many of the immensely distant quasars are receding so fast that their UV light is Doppler shifted into the visible region; they are said to have large redshifts. These cannot be compared directly with low redshift quasars whose UV emission is shifted less and still remains in the band absorbed by the atmosphere. Direct comparison is essential to find out if quasars are at cosmological distances, and the Lyman alpha line is crucial as it may provide a measure of the intrinsic brightness of quasars. So far, quasars have been disappointing cosmological objects simply because they could not be easily observed in the ultraviolet. But a recent UV observation of a low redshift quasar has imposed important constraints on cosmological models. Those measurements were made from a rocket flight and lasted only four minutes—IUE can observe for 24 hours a day.

The present knowledge of the appearance of the universe in the UV comes from balloon and rocket flights and from experiments on board the American Orbiting Astronomical Observatory satellites OAO-2 and OAO-3 (renamed Copernicus) the Dutch ANS satellite and the European TD-1 satellite. IUE is dedicated solely

to UV observations and promises an enormous improvement on the earlier, largely survey, experiments. The IUE project is itself a forerunner for the Space Telescope planned to be launched from the Space Shuttle in 1983.

There are two broad aims of IUE and these impose conflicting requirements on the instrumentation. The measurement of chemical abundances, and the study of gas motions, require a detailed study of line profiles with very high spectral resolution. But IUE should be able to detect faint sources making necessary good light collection efficiency rather than resolving power. The ability to observe for long periods also means that variable phenomena can be observed.

To satisfy these requirements, IUE carries a 1.3 m long, 45-cm diameter aperture, Cassegrain telescope which will be used exclusively for spectroscopy. No direct imaging will be made in the UV although a visible-light picture will be used for target identification. Light from the telescope is passed through two spectrographs, each containing a high dispersion echelle grating in series with a low dispersion grating; two spectrographs are needed to achieve resolutions of between 0.1 Å and 0.2 Å over the full wavelength range of 1,150 Å to 3,200 Å. For the low resolution mode, the echelle is simply bypassed, producing a spectrograph that can be used to record stars as faint as 14th magnitude with a 6 Å spectral resolution.

IUE is a joint venture between the US National Aeronautics and Space Administration (NASA), the European Space Agency (ESA), and the UK Science Research Council (SRC). The major contributor is NASA. ESA is providing the solar panels and one of the two ground stations and the SRC is supplying the UV sensitive television cameras used for spectra detectors. The observing time on IUE will be shared on a daily basis with 16 hours going to NASA, and 4 hours each to ESA and the SRC who have agreed to take 8 hour periods on alternate days.

As the telescope is in geosynchronous orbit it will be in continuous contact with the NASA ground station at the Goddard Space Flight Center and will be in view at least 10 hours a day from the ESA ground station at Villafranca near Madrid. This arrangement allows real-time operation of the telescope and reduces the training time necessary for observers. As a result, the telescope can be opened up for international use and guest observers are encouraged to use IUE in a similar fashion to ground based telescopes.

Stuart Sharrock

correspondence

Conserving uranium

SIR,—We read with interest the recent article by John Davies on this topic (1 December, page 376). However, it does contain a number of scientific errors and misleading statements which we believe it is our duty to point out.

He is incorrect in stating that the difference between the $^{238}\text{U} \rightarrow ^{239}\text{Pu}$ and the $^{232}\text{Th} \rightarrow ^{233}\text{U}$ reactions is that the first needs fast neutrons while the second can use slow ones. In fact the cross-sections for the two reactions are almost identical at all energies and therefore they have very similar breeding characteristics. The reaction $^{238}\text{U} \rightarrow ^{239}\text{Pu}$ occurs to a very considerable extent in thermal reactors. In fact about one third of the energy output of a thermal reactor arises from the ^{239}Pu produced within it and then burned *in situ*. Thus in the UK we already get between 4 and 5% of our electricity from the fission of plutonium.

The control of both thermal and fast reactors is based on the delayed neutron contribution and this is on a time scale of seconds rather than micro-seconds. The only mechanisms for a sudden increase in reactivity are loss of coolant or fuel movement, neither of which could occur faster than the control rods could move.

Turning to the $^{233}\text{U}/^{232}\text{Th}$ fuel cycle we would first challenge the statement that ^{233}U is automatically safeguarded against illegal diversion by the intense γ -rays coming from the simultaneously produced ^{232}U . This isotope does not emit γ -rays. It decays with a 70 year half-life by α -emission to ^{228}Th which does emit the γ -rays to which Dr Davies refers. Thus only after a considerable period does the γ -activity build up to a sufficient level to afford intrinsic protection.

With regard to the waste disposal problem the thorium cycle may give rise to fewer problems from the production of long lived higher activities but on the other hand much more Pu^{238} and ^{234}U is produced. The latter isotope decays to the very hazardous ^{226}Ra .

Accelerators are replacing reactors for some forms of neutron research because they can produce neutrons in short pulses and can give higher peak fluxes than a reactor. However, the mean flux from such systems up to present is lower by orders of magnitude than in research reactors.

The use of accelerators for breeding significant quantities of fuel demands machines giving outputs two or three orders of magnitude greater than the most powerful machines built so far. The breeder target is nothing less than a very major reactor in its own right. Studies of such a system at Brookhaven suggest a cost/gm of ^{239}Pu three to five times greater than that of equivalent enriched ^{235}U . The hazards of such a system could certainly not be less than that of a major reactor system.

We do not wish to give the impression that we are in any way antagonistic to the thorium cycle or to accelerator breeding. We wish merely to warn against the temptation of assuming, like the grass being greener on the other side of the fence, that problems of an alternative technology are necessarily less than those of a technology upon which one has embarked.

Yours faithfully,
A. T. G. FERGUSON
I. M. BLAIR

AERE Harwell

Transposed sentence

SIR,—It has been brought to the attention of the editors of volume 23 of *Biographical Memoirs of Fellows of the Royal Society* that certain sentences in the memoir on Professor C. H. Waddington may be read as derogatory of the work of Dr Ruth Clayton and her colleagues. This was in no way the intention of the author, but arose from an unfortunate transposition of sentences in an early draft. The author therefore wishes to amend the last six lines of page 583 to read:

"... work that Waddington had originally envisaged. A notable exception which fitted into Waddington's original concept was the work on the development of the avian lens in the group led by Mrs Ruth Clayton. The laboratory became well known for the DNA-RNA investigations which earned a world reputation for at least two of the leading workers."

We must unreservedly withdraw all inferences to the effect that Mrs Clayton's work and that of her group is anything other than of the highest class and of a deserved worldwide reputation and we whole-heartedly apologise to Mrs Clayton for the embarrassment caused to her and her

group by the unfortunate misplacing of the sentences in the article.

Yours faithfully

R. W. J. KEAY

The Royal Society,
London

Anti-Müllerian hormone

SIR,—In a recent issue of *Nature* the authors D. Tran, N. Meusy-Dessolle and N. Josso report on a testicular factor distinct from testosterone which mediates regression of Müllerian ducts in male fetuses (*Nature* 269, 411–412; 1977). Apart from the heading of their article which read "Anti-Müllerian hormone, etc." (and which caused underlining and exclamation marks by some of my merry colleagues) I do not consider it appropriate to use laboratory-born artificial words such as 'testicular anti-Müllerian activity', 'Müllerian regression' and others in a scientific communication. I wonder how this obvious jargon escaped your referee's attention*. Besides there is no 'Müllerian regression' but a regression of the outline, the diameter or so of the Müllerian duct; likewise there is neither a 'testicular anti-Müllerian activity' nor an 'anti-Müllerian hormone'. I am sure that Johannes Peter Müller (1800–1858) would agree.

Yours faithfully,

GERHARD H. MÜLLER

Universität des Saarlandes
Saarbrücken, West Germany

*It didn't, but the authors claimed that since the name had been used in all their recent publications, a change now would cause confusion.—ED.

Essential relativity

SIR,—In connection with Paul Davies' kind review of the second edition of my book *Essential Relativity* (*Nature* 22 September 1977) I wonder if you would allow me to correct one slight error. The coordinates of accelerated observers in Minkowski space, which have recently been used in the study of black holes, are elaborated on, albeit in a later section on Kruskal space. Indeed, this was one of the more significant additions to the second edition.

Yours faithfully,

WOLFGANG RINDLER

The University of Texas at Dallas

news and views

The end of the expanding Earth hypothesis?

from Peter J. Smith

ON the face of it, this is a bad day for the handful of people who support the idea of an expanding Earth, a good day for the few who oppose it, and certainly no less than an interesting day for the vast majority of Earth scientists who have been content merely to observe the progress of the expansion debate for anything up to 20 years. For on page 316 of this issue of *Nature*, McElhinny *et al.* offer the most convincing proof yet that, despite some obvious attractions, Earth expansion is nothing more nor less than a scientific blind alley. Specifically, they use the best available palaeomagnetic data to show that over the past 400 Myr the Earth's radius cannot have increased by more than 0.8%, a figure sufficiently small to exclude the very low rate of expansion (0.6 mm yr^{-1}) proposed by Wesson (*Q. Jl R. Astr. Soc.* **14**, 9; 1973) as well as the much higher rates favoured by, among others, Carey (in *Continental Drift—A Symposium*, University of Tasmania, 1958).

So that is the end of the Earth expansion hypothesis. Or is it? Before passing the death sentence it is perhaps worth making two points which may be interpreted as reasonable caution or unreasonable scepticism, depending upon one's point of view. First, history has shown that where the Earth sciences are concerned it is often dangerous to express absolute certainty. The continental drift saga alone is sufficient indication that even the most solid of foundations may crumble, especially if they are given a kick by a passing stranger. In the case of continental drift the stranger was a meteorologist, but for Earth expansion it could well be the first physicist or astronomer to delineate the historical variations, if any, in the gravitational constant. Earth scientists have been conscious of the possibility of Earth expansion ever since Dirac (*Proc. R. Soc. A* **165**, 199; 1938) suggested that

the gravitational constant may be slowly decreasing; but although convincing proof of such a decrease has never yet been produced there is still an outside chance that it will be. In that case, of course, expansion of the Earth would have to be accepted; and however hard it may be for them, Earth scientists would have no option but to re-examine their supposedly fool-proof analyses.

But if that is a possible problem for the future, there is also the more immediate question of the validity of palaeomagnetic techniques for determining the Earth's palaeoradii. As McElhinny and his colleagues point out, Eggedy (*Geofis. Pura Appl.* **45**, 115; 1960) was the inspiration for the use of palaeomagnetic data as a test for Earth expansion. His first proposal was limited to palaeomagnetic sampling sites on the same palaeomagnetic meridian, but he later (*Nature* **190**, 1097; 1961) suggested a less restrictive technique applicable to palaeomagnetic data generally. In the much improved version developed by Ward (*Geophys. J.* **8**, 217; 1963; **10**, 445; 1966) this 'minimum scatter method' involves the determination, for a single landmass, of palaeomagnetic pole positions for a series of assumed palaeoradius values. The 'correct' palaeoradius is then taken as the one corresponding to the minimum scatter of poles. Unfortunately, in the hands of Ward and others the method achieved only limited success, for at that time palaeomagnetic data were neither accurate nor extensive enough to enable any but the greatest of suggested palaeoradius changes to be detected.

That position has now changed, however, and McElhinny and his coworkers have been able to revive Ward's method in conjunction with palaeomagnetic data capable of resolving the smallest changes in the Earth's radius ever proposed. But irrespective of the quality of the raw data, is Ward's method valid in the first place? McElhinny *et al.* evidently believe that it is; but Carey (*The Expanding Earth*, Elsevier, 1976) has already argued at

great length that it is not. He concludes that the minimum scatter of poles will always occur when the assumed palaeoradius of the Earth is about equal to the present radius; so however good the basic data and however much the Earth's radius has really changed, the application of Ward's method will inevitably lead to the conclusion that the Earth has not expanded at all.

Ward assumes that although the shape of the continents must change slightly as the curvature of the Earth's surface changes, they remain constant in size and the distances and angles used in interpreting the palaeomagnetic data in terms of the palaeoradius also remain constant, when measured from a 'central point'. Ward took as the 'central point' the mean position of the rock units studied; Van Hiltten (*Tectonophysics* **5**, 191; 1967) later took the centroid of the continent; McElhinny *et al.* (who refer to the "continent keeping the same physical dimension") use both the average site location and the "approximate centre of the continental block", discovering in the process that both lead to very similar values of palaeoradius.

Carey contends, however, in a long and involved geometrical argument that during expansion of the Earth a continent will deform in such a way and to such an extent as to make nonsense of any simplified model used in the determination of palaeoradius from palaeomagnetic data. If he is completely right, palaeomagnetism presumably has no role in detecting any possible increase in the terrestrial radius. If he is right in principle but not practice (perhaps because the continental deformation is too small to invalidate Ward's model), palaeomagnetic methods will be acceptable as long as the basic data are good enough. If he is wrong, there is nothing to worry about. The fact is, however, that whether Carey is right or wrong his criticism exists and has apparently never yet been refuted explicitly. Until someone chooses to do so, there must be lingering doubt in the minds of disinterested observers. □

Progress on slime mould morphogens

from Peter C. Newell

ALTHOUGH the cellular slime mould *Dictyostelium discoideum* has yielded a great deal of information about the intercellular signalling involved during the organism's aggregation phase (the phase when the starving amoebae swarm together to form a multicellular body) there has always been the possibility that it would be just as difficult to get one's hands on the intercellular regulatory substances or 'morphogens' that are used during the later stages of morphogenetic development as it has been in more complex embryonic systems. Recent reports from two groups working with *D. discoideum* however, give strong encouragement for a more optimistic point of view.

Three classes of morphogen may be envisaged in this organism. For example, one might be concerned with controlling which of alternative pathways of development are taken, a second with inducing development in general without regard to the cell types formed, and a third with inducing the differentiation of specific cell types. In the first class is the substance that can switch developing aggregates of cells from the sorocarp (stalk and spore)-forming programme to the alternative 'slug' programme. During the slug programme stalk and spore development is held in abeyance while the organism slowly moves over the substratum in search of a more suitable place to develop. The slug is morphologically and behaviourally distinct from the alternative forms of development and is apparently formed in response to an 'awareness' by the developing aggregate of an overcrowded environment. In a recent paper by Schindler and Susman (*J. molec. Biol.* **116**, 161; 1977) the substance that induces formation of this migratory form is convincingly identified as NH_3 . This metabolite is formed in large quantity by developing aggregates as the starving cells rely on the breakdown of endogenous protein for a major part of their energy requirements. The evidence that NH_3 is the regulating agent comes from the finding that, not only does the naturally occurring agent show many of the chemical and physical properties of NH_3 but also that it is mimicked by ammonium carbonate (at a pH above 7.0) when added during the appropriate 'decision-making' period. In addition the slug stage can be omitted entirely if the NH_3 -absorbing system of glutamate dehydrogenase, NADH and α -ketoglu-

tarate is added to the young aggregates. The NH_3 seems, therefore, to be acting as a simple, metabolically produced morphogen that regulates the developmental pathway taken by the organism.

In the second class of morphogen is the inductive signal for differentiation into spores and stalk cells. From work originally performed by John Bonner (*Proc. natn. Acad. Sci. U.S.A.* **65**, 110; 1970) the regulatory substance at this level of control seems to be cyclic AMP. Cyclic AMP causes cells to transform into typical stalk cells without ever aggregating in the normal way. It is also the chemoattractive substance by which starving *D. discoideum* aggregate ready for development. However, in addition to this action there is evidence that pulses of cyclic AMP released during the aggregation process (rather than a continuous signal) are able to switch on some of the very early events of differentiation (Gerisch *et al.* *Nature* **255**, 547; 1975). Moreover, the genus *Polysphondylium*, in which cyclic AMP, although secreted at late stages of aggregation, is not a chemoattractant (this role is taken by a polypeptide instead) is also induced to form stalk cells by added cyclic AMP, (Francis *Nature* **258**, 763; 1975). The two actions of chemotaxis and stalk cell induction are therefore clearly distinct. Until very recently one was tempted to deduce from these observations that cyclic AMP has a specific effect on stalk cell induction. That this is not so is ably shown in the recent paper of Kay, Garrod and Tilley (*Nature* **271**, 58; 1978) who took, as a measure of spore induction, the number of cells induced to form the 'prespore vacuoles' (PSVs) that are characteristically formed during the course of spore maturation, rather than looking for mature refractile spores as previously. Using this criterion of spore pathway induction they found that both spore and stalk cell development was initiated by added cyclic AMP. Spore differentiation differed from that of stalk cells in that the process could not be completed to maturity under these conditions. Thus although cyclic AMP can induce differentiation, it must be a general inducer for the slime mould cells.

Important progress has also recently been made in demonstrating the existence and some of the properties of the specific cell-type inducers that can be considered as the third class of morphogen. The evidence for the idea that cyclic AMP on its own was not sufficient for stalk cell induction came from the work of Town, Gross and Kay

(*Nature* **262**, 7; 1976) who found that the proportion of cells forming stalk cells in monolayers in the presence of cyclic AMP was dependent on the cell population density. The farther apart the cells were separated the fewer stalk cells were formed. Using Cellophane membranes to separate cell layers at different densities Town *et al.* were also able to show that cells at high density on one side of the membrane were able very efficiently to help cells at low density on the other side to develop into stalk cells in the presence of cyclic AMP. The diffusible factor responsible is currently being characterised and preliminary results show that it is a small glycopeptide of 1,000 to 2,000 molecular weight whose biological activity is dependent on sialic acid, N-acetyl glucosamine and L-fucose that are present in its carbohydrate moiety (C. Town, personal communication). Using a similar technique but monitoring PSV formation to identify cells in the spore-forming pathway, Kay *et al.* described in their recent paper that although prespore cell induction is also cell density dependent (suggesting that, again, an endogenous factor is involved) the helping cells are only properly effective if present on the same side of the membrane as the cells being induced. In contrast to the stalk-specific factor the spore induction factor is not, therefore, freely diffusible through such membranes and can only act when cells are in very close proximity to each other.

The relationship between the three classes of morphogen is under active investigation. The area in which most progress has been made so far is that between NH_3 and cyclic AMP. In a model recently proposed by Sussman, Schindler and Kim (In *Development*



A hundred years ago

It is gratifying to know that at last Cleopatra's Needle has safely reached the Thames. It is proposed to moor the ingeniously constructed vessel containing the obelisk at a convenient part of the Thames embankment for some days, to enable the public to inspect it.

From *Nature* **17**, 24 January, 251; 1878.

Peter C. Newell is a Fellow and Tutor in Biochemistry of St Peter's College and a lecturer in the department of Biochemistry, University of Oxford.

and Differentiation in the Cellular Slime Moulds (Eds Cappuccinelli, P., Ashworth, J.) 31, (Elsevier/North Holland, 1977)) it was suggested that NH_3 inhibited spore and stalk cell differentiation by inhibiting cyclic AMP production. Recent work of Schindler and Sussman (*Biochem. biophys. Res. Commun.* **79**, 611; 1977) showing that NH_3 (but not NH_4^+) at physiological levels dramatically decreases cyclic AMP synthesis or release in starving cell suspensions and the work of Thadani, Pan and Bonner (*Expl. Cell Res.* **108**, 75; 1977) showing an inhibitory effect of NH_3 on cyclic AMP release by cells of the species *D. mucoroides*, suggest that this conjecture may indeed be soundly based. Whether the effect is indirect or whether NH_3 acts directly on the membrane-bound adenylylate cyclase remains to be resolved. □

Energetic oxygen ions in the magnetosphere

from D. T. Young

MAGNETIC storms are vast disturbances of the Earth's magnetosphere dissipating energy at the rate of 10^{11} – 10^{12} watts over several days. Their connection to disturbances on the Sun was realised more than 100 years ago and it is now clear that solar weather is carried to the Earth by the flow of solar wind plasma past the magnetosphere. Disturbances in the solar wind step up the vast magnetospheric dynamo thereby providing the storm's energy. But basically magnetic storms require the acceleration and transport of large amounts of hot plasma (0.1–50 keV) and it is something of an embarrassment that no one really knows where all this plasma comes from. Does it originate as cold ($\lesssim 0.1$ eV) ionospheric plasma or as relatively hot solar wind (electrons ~ 10 eV, ions ~ 1 keV per nucleon)? Given what is known about magnetic storms and their relation to the solar wind the most reasonable assumption to make is that the hot plasma also originates in the solar wind. That this is not entirely the case has been demonstrated convincingly by the recent observation during three magnetic storms of roughly equal amounts of hot O^+ and H^+ ions (0.5–16 keV) in the magnetospheric ring current (Johnson *et al. Geophys. Res. Lett.* **4**, 403; 1977).

Centred about the equatorial plane, the ring current is an enormous electric

circuit whose current is carried primarily by ions trapped in the geomagnetic field. Although the 'ring' occupies much the same region of space as the Van Allen radiation 'belts' the latter are much less dense and contribute very little net current. Magnetic storms are manifestations of the growth and decay of the ring current as hot plasma is injected into it early in the storm and is then gradually lost, primarily by charge exchange with neutral hydrogen and by scattering into the atmosphere. During storms the plasma particles penetrate deeper into the geomagnetic field and the quiescent ring current (normally centred at altitudes of $\approx 30,000$ km) shrinks down to altitudes of $\approx 10,000$ km. Although Johnson and his colleagues at the Lockheed Palo Alto Research Laboratory obtained data at somewhat lower altitudes ($\lesssim 8,000$ km), the bounce motion of the ions along magnetic lines of force enables them to sample the actual current carriers.

Together with the Lockheed group's earlier report of O^+ and H^+ ions streaming upwards out of the ionosphere (Shelley *et al. Geophys. Res. Lett.* **3**, 654; 1976) their present results have far reaching consequences for models of the aurora and storm-time ring current. Most of these rely heavily on the hypothesis that solar wind plasma enters the magnetosphere by way of the magnetotail on the night side of the Earth. Plasma acceleration and transport deeper into the magnetosphere occurs during the 'collapse' of part of the magnetotail and the accompanying conversion of stored magnetic energy into particle kinetic energy. As the heated plasma is driven deeper into the nightside magnetosphere, the more easily scattered electrons travel down magnetic field lines, bombarding the atmosphere and producing the aurora. Gradients in the geomagnetic field cause the relatively more stable ions to continue drifting in a westerly direction eventually forming the ring current. This explains why what was often called the 'proton' ring current was expected to have solar wind like composition and why it is so disconcerting to find terrestrial ions mixed in apparently large proportion. How the generally accepted hypothesis of plasma transport and acceleration from the magnetotail earthwards will be reconciled with the Lockheed group's observation of ionospheric plasma accelerated in more or less the opposite direction remains to be seen.

Johnson *et al.* obtained their data with one of a new class of particle detectors, the energetic ion mass spectrometer. Only in the past few years has it been technically feasible to build mass spectrometers with the required wide energy and mass ranges (typically

≈ 0 –15 keV per charge and 1 to >30 a.m.u. respectively) within the rigid constraints of low weight and stray magnetic fields demanded of satellite instrumentation. Perhaps the most sophisticated of the new spectrometers was first flown on the ESA GEOS satellite (Balsiger *et al. Space Sci. Instrum.* **2**, 499; 1976) while the more recently launched ISEE-A carries a nearly identical spectrometer built by the Lockheed and GEOS groups together. To appreciate that these instruments are indeed a step forward one need only consider that before 1972, plasma detectors operating between 10 eV and 50 keV were either electrostatic analysers or solid state devices used only to measure particle energy and classify their charge as positive or negative. Since solar wind plasma is known to be $\approx 95\%$ protons the energy-only detectors abetted the hypothesis that the hot magnetospheric plasma originates in the solar wind. With this assumption to go on it was not necessary to measure anything but 'protons'.

Adding to the confusion over magnetospheric plasma origins brought on by the Lockheed results are observations made with the GEOS mass spectrometer of O^{2+} and $^4\text{He}^{2+}$ inferred to be of terrestrial origin (Young *et al. Geophys. Res. Lett.* **4**, 561; 1977). We conclude that the ions are produced at altitudes $\gtrsim 20,000$ km (that is, not the ionosphere) and are accelerated during storms to energies as high as 1 keV. This result is disconcerting because it was long thought that the simplest test for solar wind plasma in the magnetosphere would be the ratio of $^4\text{He}^{2+}$ to protons. This is about 0.04 in the solar wind and, before GEOS, the terrestrial value at keV energies was thought to be orders of magnitude smaller. The $^4\text{He}^{2+}$ ion is even further compromised as a tracer because it is converted rather quickly by charge exchange to $^4\text{He}^+$. As tracers one is apparently left only with more exotic (and difficult to measure) solar wind species such as O^{6+} and ^3He . The latter has in fact been detected inside the magnetosphere by the foil collection technique.

Mass spectrometers have been something of an ugly duckling in magnetospheric research. Initially too heavy, magnetically dirty and ill suited for hot plasma measurements, they have come of age and are now invited to all the best satellite projects. The GEOS-ISEE type spectrometer will be flown on GEOS2 scheduled for launch this year, and is included on NASA's next magnetospheric research satellite the Dynamics Explorer. It therefore seems likely that energetic ion mass spectrometers will have the opportunity to supply a few answers to the many questions they have been raising lately.

Thermonuclear reactions confirmed in laser-driven fusion

from Derek Beynon

THE central concept in inertial-confinement fusion is the implosive compression of deuterium-tritium fuel to densities and temperatures where a significant number of thermonuclear reactions will occur. Throughout the world a number of experimental programmes exist to investigate the capacity of powerful lasers, relativistic electron beams and, latterly, heavy-ion beams to achieve this mode of fusion. The ideal compression should occur isentropically in an ablatively imploded pellet of cryogenically solid deuterium-tritium mixture with a final density compression of about 10^4 in order to achieve an energy 'break even' where the resulting fusion energy equals the laser input energy (*Nature* **239**, 139; 1972). Because of the power output limitations of the present generation of large neodymium glass or CO₂ lasers, which give up to 10^{14} – 10^{16} W cm⁻² on target, experiments have been limited to 'exploding pusher' targets which are glass microshells, typically 90 μ m diameter, with 1 μ m wall thickness, containing an equimolar deuterium-tritium gas mixture of density 2–2.5 mg cm⁻³. Producing a non-isentropic compression, the whole of the glass shell heats rapidly before it moves to push the enclosed gas to about normal solid densities. The resulting ion temperatures, 4–6 keV, should according to predictions allow the thermonuclear reaction



to proceed, with a very small contribution from the D–D reaction. Using such targets a group from the Lawrence Livermore Laboratory (LLL) reported at the 11th European Conference on Laser Interaction with Matter (Oxford, September 1977) that neutron yields in excess of 7×10^8 per laser shot had been achieved using the Argus neodymium glass laser facility.

Although the performance of such targets may not be very representative of ablatively driven systems they are of particular value in allowing detailed computer predictions of laser plasma interactions and magnetohydrodynamics to be checked experimentally. One question of fundamental importance which needs to be answered is whether these relatively high yields of neutrons

and α -particles are truly thermonuclear in origin or whether they are produced by fusion reactions between deuterium and tritium ions which have been accelerated to high energies within the plasma. Thermonuclear-produced particles are expected to be produced centrally in the compressed plasma whose ions have a maxwellian energy distribution at a temperature T_i (keV) giving rise to a gaussian-like energy distribution of neutrons and α -particles with a width (fwhm) of $\Delta E = 177\sqrt{T_i}$ (keV). Fusion products which are non-thermal in origin will have a different width ΔE depending on the details of the accelerating mechanisms. Early work by Rose *et al.* (*Nature* **181**, 1630; 1958) demonstrated the existence of non-thermonuclear fusion neutrons produced by the magnetic confinement device Zeta. McCall *et al.* (*Phys. Rev. Lett.* **30**, 1116; 1973) have postulated a detailed mechanism where the production of fast electrons by the resonance absorption of the laser light results in an electrostatic field on the outside of the compressed zone of the deuterium-tritium mixture. For nanosecond laser pulses a low density low temperature corona will be generated around this compressed zone before significant fast electron production occurs. As electrostatic acceleration occurs near the critical surface, where the plasma and laser light frequencies are equal, fast ions pass through the corona generating neutrons and alphas. For picosecond laser pulses, however, no low density plasma region is produced and the fast ions produce no particles. The non-thermonuclear particle production should therefore depend sensitively on the pulse characteristics and the comparisons with experiments made by McCall and others at Los Alamos would indeed seem to confirm this. The beam properties of the ions will now produce energy distributions of neutrons and alphas which differ significantly from the gaussian distribution associated with thermonuclear reactions. For example an equimolar monoenergetic D–T ion beam of energy E_i (keV) uniformly converging on a cold D–T target would result in a square distribution of neutrons and α -particles with a width $\Delta E = 150\sqrt{E_i}$ (keV).

Over the past 2 years a series of elegant experiments at LLL has confirmed the thermonuclear origin of the neutrons and α -particles produced in exploding pusher targets. The initial set of experiments (*Phys. Rev. Lett.* **35**, 1083; 1975) used a time-of-flight technique to obtain the energy distribution of the 3.52 MeV α -particles. By measuring the transit time $t = d/v$ over a flight path of length d of an α -particle of velocity v , the α -particles' energy dispersion $\Delta E = 2E(v/d)\Delta t$ is

obtained for a temporal resolution Δt . The experiment, designed so that α -particles in the range 2.6 to 3.9 MeV were magnetically deflected and detected by a scintillator, measured a dispersion consistent with a maxwellian temperature of 2.2 keV for the first laser shot and 3.0 keV for the second. This should be compared with maxwellian values in the range 1.6–3.0 keV calculated by numerically modelling the imploding microshells with a two dimensional Lagrangian hydrodynamics code. However such experiments are by no means conclusive since they assume that the α -particles are produced inside the glass microshells and consequently rely on a 0.2 MeV computed energy correction for the energy loss of the α as it passes through the compressed gas and the glass walls of the microsphere.

Consequently the Livermore group evolved an entirely novel approach to resolve the spatial distribution of the α -particles as they are born in the thermonuclear process by directly imaging the thermonuclear burn region within the target with a resolution of about 10 μ m. (*Phys. Rev. Lett.* **39**, 22; 1977). The technique, which is essentially a holographic one, was originally proposed for stellar X-ray imaging and has a number of applications in nuclear medicine (*J. Nuclear Med.* **13**, 382; 1972). It proceeds in two stages. In the first the radiation source to be imaged casts a shadowgraph (a coded image) through a Fresnel zone plate (a coded aperture) onto a suitable detector, in this case a thick cellulose nitrate film. The image reconstruction is achieved at the second stage when a negative of the shadowgraph is illuminated with coherent light to produce a Fresnel diffraction pattern which is a reconstruction of the original α -source distribution but inverted and magnified. With associated neutron yield measurements this technique showed that more than 97% of the α -particles originated in a central region, ovoid in shape, which had a diameter about one-third that of the original target.

Final confirmation of the thermonuclear origins of the neutrons and, by inference, of the α -particles has now been obtained at Livermore (*Appl. Phys. Lett.* **31**, 645; 1977) using high resolution time-of-flight spectrometry on the neutron yield. Since there is negligible interaction of the emerging neutrons with the glass microsphere and its contents, measurement of the neutron energy distribution is a particularly useful diagnostic. Using 44.5-m flight paths with fast plastic scintillation detection, a mean measured neutron energy of 14.00 ± 0.10 MeV from five experiments compares well with the expected value of 14.05 MeV. Interpreted in terms of maxwellian ion temperatures the measured energy dis-

tributions correspond to a range of temperatures from 5.2 to 6.3 keV, in excellent agreement with the predicted thermonuclear value of 5 keV.

The prevalence of chaos

from N. MacDonald

It has been known since the time of Poincaré that quite simple conservative dynamical systems, such as two coupled linear oscillators, can exhibit highly complicated behaviour. As well as periodic trajectories, which are closed curves in phase space, there are also trajectories which, loosely speaking, fill up volumes in phase space. The rather special nature of conservative systems limits the number of physical contexts in which this behaviour can be manifested. (For some interesting examples see Henon & Heiles *Astr. J.* **69**, 73; 1964; Dunnett, Laing & Taylor *J. math. Phys.* **9**, 1819; 1968.) Analogous behaviour has been observed in recent years in a number of nonlinear models of physical systems which are open or dissipative, rather than conservative. This is leading to reappraisal of our understanding of these systems.

The best known example is that put forward by E. N. Lorenz (*J. atmos. Sci.* **20**, 130; 1963). He starts from the partial differential equations for flow in a layer of liquid of uniform depth with a constant temperature difference maintained between the upper and lower surfaces. These have a steady state solution with zero flow. As the temperature difference is raised this solution becomes unstable and convective flow solutions replace it. Lorenz examines one particular mode of convective flow, and uses a truncated Fourier expansion to reduce the problem to one involving three coupled ordinary differential equations. The variables are the intensity of convective flow, the temperature difference between ascending and descending currents, and a quantity measuring the departure from linearity of the temperature gradient across the fluid layer. Lorenz finds a second instability, beyond which the simple smooth convective flow is replaced by an extremely complex and unpredictable flow. The mathematics of this situation have become popular in the past few years, and progress in understanding the nature of the complex flow is reviewed in the proceedings of a seminar

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The blind fishes of Persia

THE note published in *News and Views* (**264**, 113; 1976) on the blind cave fishes of Persia mentioned the discovery of the two cavernicolous forms *Noemachilus smithi* (Cobitidae) and *Iranocypris typhlops* (Cyprinidae) in the same well-like outlet. This is of great interest for the zoogeographical and etho-ecological interpretation of the possible mechanisms which led epigeic fishes to colonise subterranean waters and evolve underground into isolated homogeneous populations composed entirely of eyeless and depigmented individuals. These phenomena of regressive evolution have been well studied on several populations inhabiting caves (Thinès *L'évolution régressive des Poissons cavernicoles et abyssaux*, Masson, Paris, 1969), but little, if any, specific attention has been given to the case of cavernicolous forms found in wells and artificial outlets. It is therefore fortunate that Mr Smith was able to discover this new *Noemachilus* form in association with the previously discovered *Iranocypris* (Brunn & Kaiser *Dan. Scient. Invest. Iran*, Munksgaard, Copenhagen, 1943). However, the coexistence of two distinct regressed species in the same well has been observed before. In 1962, Mees found two different blind and depigmented Teleosts, *Milyeringa veritas* (Gobiidae) and *Anommatophasma candidum* (Synbranchidae) in two wells depending on the same subterranean system and located about 10 miles apart, near Yardee Creek Station, North West Cape, Australia (Mees *J. R. Soc. West. Austr.* **45**, (I), 24; 1962).

The coexistence of distinct cavernicolous forms in a same biotope other than a cave, raises the question of the exact nature and extent of their habitat. Underground waters can be traced with sufficient precision in many karstic systems (the system of sinks, underground caverns and streams found in limestone areas), whereas water sheets feeding wells or pumping sites do not lend themselves to similar descriptions. In addition, such phreatic water tables cannot be explored by speleologists, so that the only topographical data are those furnished by geological surveys. Thus, biospeleologists have had to limit themselves to inference as far as the

habitat is concerned. The only sound hypothesis about regressed fishes found in wells and other artificial water outlets is that these forms normally live in the phreatic sheets and are occasionally attracted to zones exposed to light by organic remnants of animal or human origin. The fact that fishes of the same species are sometimes observed in wells separated by a great distance is, in my opinion, the strongest argument in favour of this view. Mees reported the presence of the free-swimming *Milyeringa* in only two of the three wells he visited (*Milyering*, Kudmurra and Tandabiddi, the last halfway between and some 10 miles from the other two), whereas the eel-like *Anommatophasma* was found in all three sites. The cases of *Iranocypris typhlops* and of *Noemachilus smithi* seem similar to that of *Milyeringa veritas*. The ecological conditions in which these fishes live are moreover similar to those reported as early as 1904 by Goeldi for *Phreatobius cisternarum* (Goeldi *Rept. 6th Int. Zool. Congr. Bern*, 549; 1904), a very peculiar catfish belonging to the family Trichomycteridae which he found in a cistern of Marajo Island in the mouth of the Amazon and which was rediscovered recently (Delamare, personal communication). I have collected specimens of the blind Clariidae *Uegitglanis zammaranoi* in the canalizations of the Ischia-Baidoa pumping station (Somaliland) some 60 miles from the well of Uegit, where the first specimens were discovered in 1923 by Gianferrari (*Atti. Soc. Ital. Sc. Nat. Mus. Civ. Stor. Nat. Milano*, **62**; 1923). To the present day, this distance is by far the greatest to have been reported for the geographical extension of a cavernicolous fish not dwelling in a cave (Thinès *Rev. Zool. Bot. Afric.* **57**, 117; 1958). The case of *Noemachilus smithi* can thus be considered as a typical instance of an already well-known phenomenon, whose meaning for biospeleological research is clear.

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on turbulence held at Berkeley in 1975 (Springer Lecture Notes in Mathematics No. 615; 1977.)

The conditions for this new instability are physically unattainable in this experiment. However, H. Haken has

pointed out (*Phys. Lett.* **53A**, 77; 1975) a remarkable parallel between the Lorenz model and a classical approximation to the dynamics of a single mode laser. In this case the three variables are the field strength and polar-

isation of the radiation, and the inversion of the atomic levels. The second instability can be attained if the laser cavity loss is high enough. Haken identifies this instability with the onset of undamped spiking of the laser output.

These models are purely deterministic, being set up in terms of ordinary differential equations. Yet the solutions behave in an apparently random way. The term 'chaos' has been appropriated to refer to this intriguing feature of nonlinear dynamical systems of three or more dimensions. A beginning has been made in the classification of different types of chaotic flow, and the determination of the conditions under which they occur. In a fascinating series of papers (*Z. Nat.* **31a**, 259, 1168, 1664; 1976; **32a**, 607; 1977) O. E. Rössler outlines some of the taxonomy of chaotic flows, and sketches a number of potential applications.

To visualise one possibility, think of a conical spiral seashell with a central pillar, and imagine a line winding out along the expanding spiral until it reaches the mouth of the shell, then tucking itself into the pillar and re-injecting into the narrowest coil. The equivalent behaviour is impossible for the trajectory of a dynamical system in two dimensions, because it would require that the trajectory cut itself at a point which is not a point of equilibrium. This kind of spiral trajectory could of course be periodic. The essential new thing about chaos is that for some sets of nonlinear equations all such periodic trajectories are unstable. The majority of trajectories then behave as if repelled, not only by the unstable focus at the point of the shell, but by each of the unstable periodic trajectories. Each time a trajectory re-injects to somewhere near the point it emerges in a different direction.

One may invent other varieties, for example two unstable foci with reinjection from the outside of each spiral to an inside coil of the other. H. Haken and A. Wunderlin (*Phys. Lett.* **62A**, 133; 1977) have given a simple geometric picture of the Lorenz-laser chaotic flow.

Rössler's extensions of the applications of chaotic flows have mainly been to schematic models of chemical reactions, involving oscillating and switching components. He has also discussed the relevance of this topic to the Rashevsky-Turing model of morphogenesis as controlled by chemical reactions and diffusion between cells. In a recent issue of this journal (*Nature* **271**, 189; 1978) Rössler and K. Wegmann propose the application of these methods to the Belousov-Zhabotinskii oscillating reaction (for a recent review see Tyson *The Belousov-Zhabotinskii Reaction* (Springer Lecture Notes in

Biomathematics No. 10; 1976)).

When this reaction is studied in a well-stirred solution, oscillations of the chemical concentrations, with well-defined period, can be detected by colour changes in an appropriate indicator. Also when the solution is in a shallow dish and unstirred, waves of colour change can be observed on the surface. A. Winfree suggested (personal communication to Rössler) that some of his observations on these waves might indicate symptoms of chaos. Rössler and Wegmann now report observations of the electrochemical potential in a well-stirred Belousov-Zhabotinskii reaction. This shows irregular spiking over times of the order of an hour, with about three spikes every 2 minutes. They suggest that this may be related to a particular type of chaos, which they illustrate by computed solutions of a very simple set of equations

$$dx/dt = -y - z$$

$$dy/dt = x + 0.55y$$

$$dz/dt = 2 - 4z - xz$$

Even this apparently innocent set of equations, in which there is only one nonlinear term, have rich possibilities. They can exhibit the 'seashell' type of chaos as well as the type which Rössler and Wegmann consider may be present in the Belousov-Zhabotinskii experiment. The authors point out that simultaneous measurements of two or more quantities can give a much more critical test of their hypothesis, and comment briefly that they have some promising results, plotting the electrochemical potential against the potential of an electrode sensitive to bromide ions.

Other authors (Schmitz, Graziani & Hudson, *J. Chem. Phys.* **67**, 3040; 1977) have reported data on the well-stirred Belousov-Zhabotinskii reaction consistent with a different type of chaotic flow while L. F. Olsen and R. Degn (*Nature* **267**, 177, 1977) have presented evidence for chaotic flow in the horse-radish peroxidase reaction. It is thus becoming apparent that even without the manifest complications brought in by considering diffusion as well as reactions, these systems reveal a rich variety of phenomena. This will undoubtedly stimulate much further work, both theoretical and experimental, on chemical oscillations and on their possible bearing on biological problems.

Natural fission reactors—Oklo style

from S. A. Durrani

A Technical Committee Meeting (Experts Group) on Natural Fission Reactors was held at Paris on 19–21 December 1977. It was organised by the International Atomic Energy Agency (IAEA) in collaboration with the French Atomic Energy Commission (CEA).

AFTER 5 years of investigation into the Oklo phenomenon, it is no longer a question of why it happened but why it should not have happened all over the world. Several researchers have been hunting for clues at likely places but as yet no other natural fission reactors have turned up.

The Oklo phenomenon (see *News and Views* **256**, 264; 1975), was first discovered by French scientists in 1972 when they established that a self-sustained chain reaction had occurred in a uranium mine, some 1,800 Myr ago, at Oklo in the Republic of Gabon in west equatorial Africa. The higher natural abundance of ^{235}U , namely over 3%, prevailing at that time (more than two half-lives ago for the isotope), the moderation of the fission neutrons by soil water (making it more likely that they would initiate new fission reactions), and effective absence of neutron-absorbing 'poisons', constituted favourable conditions for the occurrence of a self-propagating fission reaction. Many of the fission products, in proportions exactly the same as those obtained in today's man-made reactors, still lie there as evidence of the long-ago activity. That in itself was an important discovery, with far-reaching implications for the storage of nuclear waste.

The Paris meeting of the Technical Committee on Natural Fission Reactors (Experts Group), convened jointly by the International Atomic Energy Agency (IAEA) and the French Atomic Energy Commission (CEA), was the first of periodic conferences on the subject recommended to be held by a permanent international working group on Natural Fission Reactors (NFRs) set up under the auspices of the IAEA following the 1975 conference at Libreville in Gabon. Over 40 papers were presented on topics ranging from the geology and petrology of the Oklo reactor materials to isotopic chemistry, radiation damage studies of samples from a reaction zone and its surroundings, reactor physics

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models of the NFRs, and the role of organic materials in concentrating the initial uranium ore deposits.

The opening address was delivered by R. Naudet (CEN, Saclay), the scientific secretary of the meeting and since 1972 the director of the 'Franceville Project' established by the French CEA to promote and coordinate the international investigation of the NFR phenomenon. He reported on the 5 years of exploration of the Oklo site and of the international ramifications of the project. In the papers that followed, the emphasis was on natural fission reactors in the plural—not only at Oklo but elsewhere in the world. The development of the phenomenon was traced (or imagined) in both time and space. Investigations have shown that there was not just one reactor at Oklo: at least six 'reaction zones' over a relatively small area (a few thousand square metres) have been identified. A question exercising not a few minds was whether one of these was a 'primary' reactor and the others either secondaries or its lineal descendants. The reactors, like reproducing organisms, seem to have been 'propagating' themselves. Naudet, in true detective story style, termed this the 'Oklo mystery'. The age of the phenomenon has now been put back from $\sim 1,800$ Myr to nearer 2,000 Myr; the duration of the entire drama at Oklo, from the first spark to the last smolderings of the atomic fuel has also been extended from $\sim 500,000$ years to ~ 2 (or perhaps several) Myr.

A systematic search for other natural fission reactors is now being conducted elsewhere in the world, as described by K. E. Apt (Los Alamos Scientific Laboratory). Apt and his colleagues have analysed high-grade pre-Cambrian uranium ores from Australia, Canada, South America, Africa and the USA, for tell-tale isotopic variations in uranium and its fission products. No isotopic anomalies, indicating criticality, have so far been discovered in the samples from any of those sources. But, as was evident during discussion, everyone now expects another natural fission reactor to be discovered in some part of the world sooner or later. The hunt is on.

Amongst the papers on physico-chemical aspects of the phenomenon were those on estimation of the reactor temperature (~ 280 – 400°C) by P. Holliger and colleagues (CEN, Saclay) and R. Vidale (Los Alamos Scientific Laboratory); gauging the adverse effect of intense radiation on the thermoluminescence properties of Oklo materials inside the reaction zone by S. A. Durrani and colleagues (University of Birmingham); problems of criticality and reactor control in Oklo and other NFRs by R. Naudet; and geochemical study of insoluble organic

material (kerogene) of the Oklo uraniferous ore by M. Vandenbroucke and colleagues (Institut Français du Pétrole).

A final round table discussion focused on the relevance of the Oklo experience to present-day problems of nuclear waste storage. G. A. Cowan (Los Alamos Scientific Laboratory) reported evidence for the remarkable stability of uraninite (UO_2) grains at the Oklo reactor site. He estimated fractional loss rates of 10^{-10} yr^{-1} for elements such as uranium and plutonium. Even for the most volatile

elements, the loss rates during the operating period of the reactor were unlikely to have exceeded $\sim 10^{-6}\text{ yr}^{-1}$. D. G. Brookins (University of New Mexico) demonstrated that many of the fission products (the rare earths, for example) had hardly migrated from the host pitchblende and its associated gangue over the past $\sim 2 \times 10^9$ yr. These observations greatly strengthen the case for the storage of long-lived nuclear waste products (such as plutonium) in tectonically stable geological repositories, possibly after incorporating them in synthetic uraninite matrices. □

Telescopes of the future

from Edward Kibblewhite and John Whelan

The ESO Conference on Optical Telescopes of the Future was held at CERN, Geneva on 12–15 December, 1977.

CONTINUOUS improvement of detectors has enabled astronomers to use the light collected by telescopes more efficiently and to observe fainter objects in shorter times without building bigger telescopes. L. Woltjer (ESO, Geneva), in opening the conference, pointed out that the 'collecting power' of telescopes (area of mirror times quantum efficiency of detector) has doubled every 7 years since 1910. Since the next generation of detectors will approach quantum efficiencies of unity in the near future, the only way to improve collecting power is to build telescopes of bigger effective mirror area.

However, I. King (ESO), R. Ekers (Gröningen) and M. Disney (University College, Cardiff) arguing from different standpoints, showed that, when observations are background-noise limited, the important scaling factor for signal-to-noise detection efficiency is diameter of telescope (D)/seeing disk size (S). Only high-resolution spectroscopy, which is photon noise limited, scales at D^2 . Some parts of the conference were concerned with increasing D and others with decreasing S . The cost of a telescope system (including dome, computer and so on) using current design scales as D^γ where γ was said to be between two and three; to build large telescopes therefore requires new designs to keep the cost down.

Two telescopes, which are to come into operation shortly, showed that low mirror weight can give great savings in

cost. J. Ring (Imperial College, London) described the new SRC 3.84-m infrared telescope being built in Hawaii. This uses a very thin mirror and a light mounting and cost £2.5 million, one-fifth to one-half the cost of a conventional telescope of the same aperture. P. Strittmatter and colleagues described the SAO/University of Arizona multiple-mirror telescope (MMT). This consists of a rigid frame holding six 1.8-m telescopes which converge the light to a common focus. Each telescope can be moved individually under computer control, and is aligned by looking at a bright star and analysing the image at the focus by computer. With all telescopes aligned, a 0.8-m telescope in the middle of the array, which also acts as a guider, produces a set of parallel laser beams which maintain the alignment of the six telescopes with respect to each other. The MMT will produce $\frac{1}{2}$ arc s images over a 1 arc min field of view, though it is primarily to be used for on-axis work (about 80% of the observations at a typical observatory are on-axis). The structure is alt-az mounted and the whole building rotates. The MMT, which contains many important innovations in telescope design, is said to cost \$8 million and has an equivalent aperture of 4.5 m.

Kitt Peak presented four designs for the 'next generation telescope' (NGT), each providing a 25-m equivalent aperture. A rough cost of \$10⁹ was mentioned—the cost of the Very Large Array (VLA). One design, the 'Shoe', involved a rotatable sector of a large spherical mirror, like part of the Arecibo radiotelescope, the spherical aberration being corrected by auxiliary optics. Other proposals were an enormous steerable dish made out of a mosaic of mirrors, an MMT design

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with six 10-m telescopes, and the 'singles array', in which six 10-m telescopes would feed a central coude focus (see picture). The engineering problems of building a steerable telescope of 25-m diameter were felt to be serious; furthermore the large 'plate scale' (mm per arc s) makes the telescope incompatible with modern detectors. Simultaneous guiding of several smaller telescopes does not seem to be a problem; a suggestion to use accurate gyroscopes to achieve this was made.

Various siderostat telescopes were proposed which have a fixed telescope fed by a larger flat. A. Meinel (Optical Sciences Center, Arizona University) described one with a 45° flat and R. Bingham (Royal Greenwich Observatory) a neat alt-alt design. W. Richter (ESO) proposed a novel telescope which used a giant windmill, both to protect the undomed telescope from wind vibration and also to generate 300 kW of power! A continuing theme throughout the discussion, and emphasised particularly by E. Becklin (NASA, Hawaii) was the need to provide for adequate infrared capability.

At present, observing time on large telescopes is heavily oversubscribed. Disney has, for some years now, proposed incoherent arrays of telescopes in which information is collected by detectors and combined afterwards. He suggested a group of telescopes all identically instrumented which could either all point at one object or act independently. The availability of essentially noise-free detectors now makes this scheme very attractive from an astronomical point of view.

The importance of obtaining greater efficiency by improving the effective seeing disk size was not overlooked during the conference. One uncertainty concerning large (≤ 10 -m) mirrors is whether temperature effects across the mirror would worsen the seeing. Meinel claimed this was already significant. For the proposed space telescope the 'seeing' disk will be about one-tenth that of a good ground-based site and it is this feature which allows it to go so faint. Interesting papers were given by F. Dyson (Princeton University) and J. Hardy (ITEK Corp., Lexington) on the use of active optics to compensate for seeing by deforming the telescope mirror itself or the wavefront near the focus. Hardy showed data from a 1.5-m telescope in which active optics had reduced the seeing disk by a factor of two or three. The technique is probably restricted to bright stars.

The remainder of the meeting was devoted to ground-based optical interferometric techniques for obtaining ex-

Rift Valley fever

from Arie J. Zuckerman and David I. H. Simpson

RIFT Valley fever or enzootic hepatitis is a severe viral infection which primarily affects sheep and cattle causing many deaths in pregnant and newborn animals. The disease occurs naturally only in Africa and by 1912 it was recognised in the Rift Valley in Kenya. The infection is mosquito-borne and the virus, a member of the Bunyamwera group of arbovirus was isolated and identified in 1931. It was then also recognised that many infections occur in man, particularly by direct contact with sick animals or carcasses. Laboratory infections have also been reported. In 1950-51 an epidemic of the disease occurred in South Africa during which it is estimated that 20,000 persons became infected and 100,000 sheep and cattle died from the disease. Another severe epidemic occurred in the central regions in Southern Africa in 1975 (Van Velden *et al.* *S. Afr. med. J.* **51**, 867; 1977).

In October and November 1977 an outbreak of Rift Valley fever occurred for the first time in Egypt in the Nile delta. The number of human cases has been estimated at 10,000-20,000 with 70-80 deaths. In the areas most affected in the provinces of Sharqia Qalyub and Giza as many as 70% of the population were infected (*WHO Wkly. Epidem. Rec.* **53**, 1; 1978). The disease in man usually follows a transient febrile course with severe headache and bodily pains. Complications include haemorrhage, encephalitis and involvement of the eye with macular degeneration and temporary or permanent blindness. Haemorrhage generally indicates a

poor prognosis and autopsy of cases revealed necrosis of the liver associated with haemorrhage, tubular necrosis of the kidneys, pulmonary congestion and haemorrhages in the stomach and colon.

Experimentally lambs are highly susceptible and may die from massive necrosis of the liver within 36 h of infection. Mice die with hepatitis within 3 days, and other laboratory rodents are readily infected. Monkeys develop a mild fever. The pathological lesions are principally those of massive hepatitis in lambs and focal lesions of the liver in older sheep. There is often damage to kidneys, and there may be haemorrhages in the intestines and elsewhere.

In the entomological investigations undertaken in the affected areas in the recent epidemic in Egypt virtually only *Culex pipiens* was found and indeed the virus was isolated from this species of mosquito. People using mosquito nets or otherwise avoiding exposure to mosquitoes at night were apparently not affected. As before, contact and slaughter of sick animals is considered to have caused at least some human cases. While it will be extremely difficult to establish the exact origin of the outbreak it may have resulted from the smuggling of camels (*WHO Wkly. Epid. Rec. op. cit.*). This epidemic illustrates once more that viral infections know no boundaries.

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tremely high spatial resolution. Hanbury Brown (University of Sydney) gave a critical review of intensity versus Michelson interferometry and concluded that, although his intensity interferometer had achieved all its aims it was probably not competitive with a Michelson for faint objects. This is mainly because the bandwidth of a Michelson is so much greater than that of an intensity interferometer. A. Labeyrie (CERCA, St Vallier-de-Hiery) has made great progress in this area and showed pictures and results of his Michelson interferometer which uses two 25-cm telescopes separated by 20 m. He also described the construction of novel and cheap 1.5-m tele-

scopes to be used in a much more ambitious set-up. C. Townes (University of California, Berkeley) described a heterodyning interferometer for use in the infrared. Several papers discussed various aspects of speckle interferometry which now appears to have become a standard technique.

A. Boksenberg (University College, London) gave a comprehensive and well-organised review of trends in detector development and some astronomical stories were related by J. Greenstein (Caltech). We were also treated to L. Goldberg's (Kitt Peak) visit to China and a tour of the 400 GeV accelerator, which rounded off an extremely successful conference.

review article

Plasma waves in the magnetosphere

D. J. Southwood*

A large variety of plasma wave phenomena are seen in the Earth's magnetosphere. Attempts at the theoretical explanation have had some successes, including wave induced loss of radiation belt particles and the Kelvin-Helmholtz instability source of geomagnetic pulsations. But there are also areas where theory needs more development: for example, on the ion wave turbulence seen on auroral magnetic flux tubes, the role of anomalous resistivity and the origin of the terrestrial kilometric radio waves.

THE Earth's magnetosphere provides a vast variety of plasma wave phenomena over nine decades of frequency ranging from hydromagnetic waves at 1 mHz, whose field at ground level was first detected more than one hundred years ago¹, up to the radio waves near 1 MHz which make the Earth at times the brightest radio planet in the solar system².

The plasma in the magnetosphere is collision-free and 'anomalous' transport effects may be caused by plasma waves and turbulence. Some waves, by contrast, are a secondary effect, but they are still interesting for the information they give about the state of the plasma that supports them. In fact because space exploration has provided much knowledge of the variety of background conditions, the magnetosphere has been a good testing ground of collision-free plasma theory³. There have been notable successes but also some surprises. Here we review some recent examples from both classes.

The background plasma

The plasma distribution in space and energy is crucial in determining what waves occur in a plasma and it is appropriate to start by a discussion of this.

The basic distributions of plasma in the magnetosphere can be rationalised by recognising the dominant role of solar wind driven convection. Because the charged particle Larmor orbits about **B** are much smaller scale than any gross feature except near the neutral sheet in the tail and at the magnetosphere boundary, everything is ordered as illustrated in Fig. 1 by magnetic flux tube. To a first approximation, any two particles remain gyrating about and bouncing back and forth along the same tube as each other throughout the convective motion. In fact we can think of the flux tubes themselves as moving, carrying particles along with them.

The magnetosphere forms an obstacle to the solar wind plasma streaming from the sun and a bow shock forms. Between the shock and the magnetopause boundary is the turbulent magnetosheath. Much of the sheath plasma is deflected around the magnetosphere but direct entry occurs in the cusp region and remnants of this plasma are swept back into the high latitude tail as the plasma mantle. Convection over the polar caps is tailwards, so that in the tail the plasma flows inwards towards its centre plane from both sides. In the centre of the tail is a weak field region, the neutral sheet, with a strong current. Field lines are 'broken' here by the reconnection process and emerging from this region with a sunward flow is a hot plasma with temperature 1–2 keV and density of a few particles cm⁻³ called the plasma sheet. This hot plasma trapped in the Earth's

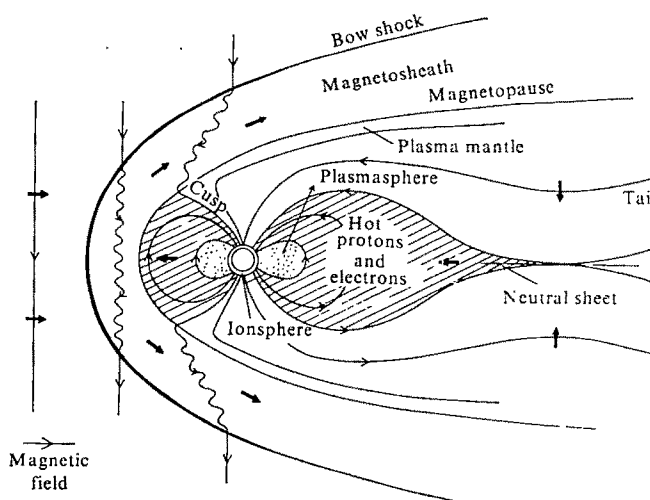
magnetic mirror field geometry flows inwards and drifts around the Earth to become the 'ring current' plasma, so called because of the depression in magnetic field it produces on the ground at the Earth. Throughout the ring current/plasma sheet the average proton energy exceeds the electron energy by about a factor of 2.

Within this region, in an ideal picture, is a region corotating with the Earth, the plasmasphere. This contains relatively cool plasma (temperature < 1 eV) with high density ($\sim 10^{-3}$ m⁻³) which is in equilibrium with the ionosphere. In a steady convection pattern ring current protons can penetrate the plasmasphere while hot electrons do not. This is because hot particles, as well as their convective 'flux tube' motion, have drift that is dependent on energy and charge.

As well as the ring current and plasmasphere populations the inner magnetosphere also contains the very energetic particles (energy up to MeVs in the inner zones) which constitute the Van Allen radiation belts. This energetic tail of the distribution plays little part in convection though their distribution is understood largely in terms of their injection and diffusion inwards by fluctuations in the convection system.

Several natural features of the convection system deserve

Fig. 1 An idealised view of the noon-midnight meridian magnetosphere. Broad arrows indicate the convective motion of flux tubes. The whole flux tube of plasma down to ionospheric heights moves together except in a region on the dayside magnetopause and in the tail neutral sheet where flux tubes are broken by a process called reconnection. The diagram is not to scale. The tail is very extended (~ 500 – $1,000 R_E$ (Earth radii)).



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note at this point. Because flux tubes move as a whole, stresses are transmitted from the equatorial regions of field lines to the ionosphere and this is done by field aligned currents, 'Birkeland' currents, which may be carried by beams of particles. Birkeland currents have been detected near the outer boundary of the plasma sheet on high latitude field lines which map to the neutral sheet region, and it is also widely believed that the primary particles providing the discrete auroral displays (thin bright arcs) originate from this region. A second layer of Birkeland current oppositely directed to the high latitude set is found at lower latitudes and maps to the region occupied by the hot ring current/plasma sheet plasma. Plasma sheet electrons are regarded as the source of the steady 'drizzle' of electrons into the ionosphere that gives rise to the relatively structureless aurora, the diffuse aurora seen equatorward of the thin arcs.

The next point to note is that the convection system naturally provides both a heating of the hot plasma as it convects Earthward on the nightside and an increase in pressure anisotropy (pressure perpendicular to \mathbf{B} > pressure parallel to \mathbf{B}). Finally a curious feature of energetic particle motion in the convection system is that the protons which best penetrate the plasmasphere are those with 10–20 keV energy, so that just inside the plasmasphere proton distribution functions occur with an absence of lower energy particles.

The convection can thus generate strong currents parallel to \mathbf{B} , particle beams, pressure anisotropies and particle distributions with a 'hole' at low energies (that is non-monotonic energy distributions). It also provides steep spatial gradients in particle distribution. Any of the preceding effects can provide a source of free energy which may drive instabilities or a steady spectrum of plasma waves. Often one envisages a quasi-steady situation where a relatively unchanging wave amplitude is sustained by convection or similar process providing energy at the rate wave energy is lost by some dissipative process such as bad ionospheric reflection.

Lastly note that convection is in fact 'gusty'. The most obvious manifestation is the substorm phenomenon when the auroral pattern breaks up, reconnection in the tail increases and convection and all its attendant phenomena intensify.

The above gives an outline of how the magnetospheric plasma distributions are set up, and as further background it is useful to note here some of the features of the characteristic frequencies that occur in plasma wave theory. The frequency at which protons gyrate about the magnetic field \mathbf{B} , the gyro- or cyclotron frequency, f_p^+ is proportional to the field B ($\approx 0.015 B$ Hz with B in gamma, $1\gamma = 10^{-9}T$) and in the equatorial plane ranges from a fraction of a Hz in the weak tail field to about 10 Hz in the inner magnetosphere. The corresponding electron cyclotron frequency, f_p^- ranges from a fraction of a kHz to a few tens of kHz in inner regions. The plasma frequency, f_p , the frequency at which electrons naturally oscillate about the ions in a cold plasma, is proportional to the square root of the electron density, n . In fact $f_p = 9\sqrt{n}$ Hz where n is the number of particles m^{-3} . f_p generally exceeds the electron cyclotron frequency but by typically less than an order of magnitude. In some regions off the equator, in particular a few Earth radii (R_e) above the auroral region ionosphere where much of the auroral kilometric radiation is believed to originate, the electron cyclotron frequency exceeds the plasma frequency.

This then sets the scene. The magnetosphere has hot and cold plasma populations, very largely collisionless and often far from thermodynamic equilibrium. Characteristic frequencies range from less than a Hz to hundreds of kHz (near the ionosphere). Waves are observed over the whole interval 1 mHz to about 1 MHz thus entirely containing the above range.

Waves around the electron cyclotron frequency

The word 'around' in the section title is to be interpreted loosely; the waves we look at in this section have frequencies from a few hundred Hz up to tens of kHz. We start by looking at frequencies below the electron cyclotron frequency. In a

cold plasma in this frequency range the wave that propagates is called the 'whistler' mode; the signal is in the acoustic band and recordings of signals which have come through the magnetosphere in this mode exhibit a characteristic whistling sound when played back through a tape recorder. Many whistler signals can be detected on the ground and some have an atmospheric source in lightning discharges.

It was recognised early on that whistler mode signals could strongly interact with energetic electrons⁴. These fast electrons see the wave signal doppler shifted to their cyclotron frequency and a resonant interaction results. Even small amplitude waves can produce large orbit perturbations. Most significantly the pitch of the resonant particle's helical orbit along the magnetic field is altered and in the presence of a spectrum of waves a nett 'pitch angle diffusion' results. Particles with smaller pitch angles mirror further down the flux tube closer to the Earth and thus have an increased chance of being lost by collision in the atmosphere.

In the interaction electrons lose energy to the whistlers as their pitch angle is reduced and as a result a distribution with a preponderance of particles with large pitch angles is unstable to whistler waves. Such a pitch angle (and pressure) anisotropy is produced naturally by convection. Kennel and Petschek⁵ pointed out that a steady state could be attained on a flux tube. Convection provided a source of particles while loss is by pitch angle diffusion. Waves gain energy because of the inherent instability but lose energy in bad ionospheric reflection. Development of a self-consistent theory still continues⁶.

In the magnetosphere it is interesting to note that two different kinds of whistler noise are commonly seen at high altitude. Outside the plasmasphere there is an emission called 'chorus'. Again named for its character, when replayed acoustically chorus resembles the dawn chorus of birds; the emission is sporadic, fluctuating on a time scale of seconds. Inside the plasmasphere the characteristic emission is a featureless 'plasmaspheric hiss' which effectively fills the high density region but is believed to be generated by cyclotron resonant interactions with >30 keV electrons near the outer edge⁷ of the plasmasphere. Detailed tests of the pitch angle diffusion theory have been made using typical wave fields and observed particle fluxes⁸ and it seems the basic mechanism is established. It is not established why chorus is structured in the way it is. The structure may well be allied to the nonlinear mechanism whereby signals from ground transmitters in the same frequency band generate sideband signals. Recently the Stanford group have shown that similar effects are achieved by very high harmonics of 60 Hz power line radiation entering the magnetosphere⁹ and have also shown that chorus occurrence may be related to power line radiation¹⁰.

An example of chorus and hiss occurrence is shown in Fig. 2 which shows data¹¹ from the Explorer 45 spacecraft. The spacecraft leaves the plasmasphere at 0424 UT (universal time). The hiss apparent in the channels below 1 kHz disappears at about this time and shortly afterwards chorus emissions appear in the 3.11 kHz channel for about an hour. It returns rather intermittently for about an hour before the re-entry of the spacecraft into the plasmasphere.

Also shown are emissions above the electron cyclotron frequency. In contrast to the whistler mode these signals have negligible magnetic signal and thus are electrostatic. First reported in 1970¹² these signals are specifically hot plasma phenomena which occur in bands between harmonics of the electron cyclotron frequency. They are regarded as responsible for pitch angle diffusion of the 1–10 keV electrons whose precipitation into the atmosphere provides the diffuse aurora. Simultaneous particle data from Explorer 45 (not shown) shows that the flux of these electrons is higher outside the plasmasphere than within it. The emissions are strongest in the band centred on $3f_p^-/2$. This is the characteristic band but near spacecraft apogee noise is present up to the $11f_p^-/2$ band. The theory of these waves and the associated precipitation is far short of the developed state of the whistler instability.

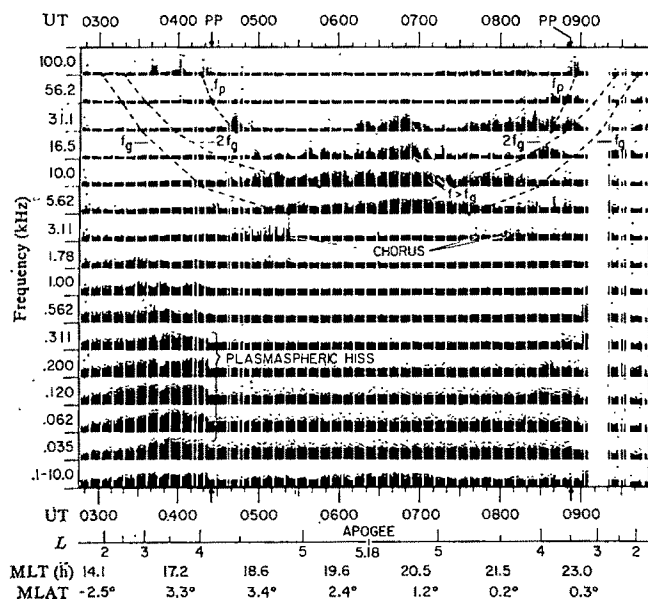


Fig. 2 Exp 45 (S³-A) spacecraft wave electric field data, orbit 214 day 23–23 January 1972. There are 15 narrow band channels and one broad band. Channel output is approximately proportional to the logarithm of the amplitude on a scale that varies from 2 $\mu\text{V m}^{-1}$ to 20 mV m^{-1} . Vertical lines are 64 s averages while dots indicate peak amplitudes in the same period. f_g and f_p indicate electron gyrofrequency and plasma frequency. PP marks exit from the plasmasphere. L indicates the radial distance of the equatorial point of the magnetic field line through the spacecraft (in R_E). UT is essentially Greenwich mean time and MLT is local time. MLAT is latitude with respect to the geomagnetic equator. From Anderson and Maeda¹¹.

It is more complicated than the whistler instability discussed above for a variety of reasons. Whistler waves are relatively fast and pressure effects are unimportant. Here they are all important and complicated because wavelengths are comparable to the mean Larmor radius of the electron population. It is now agreed that waves are driven by pitch angle anisotropy of electrons and also that a weak cold background plasma is important¹³ and it was shown that the growth rate should peak just below the upper hybrid frequency, $(f_p^2 + f_g^2)^{1/2}$ (refs 14 and 15). The fact that observation shows that the waves just above f_g^- seem to be preferentially excited is resolved by noting that the group velocity is smaller for lower frequency waves and thus they can stay longer in the generation region.

The waves are intense with amplitudes of 1–10 mV m^{-1} when compared with a background DC electric field associated with convection of a fraction of 1 mV m^{-1} in the magnetospheric equatorial plane and estimates¹⁶ suggest that their effect on particles they interact with are dramatic; 10 mV m^{-1} amplitudes are estimated adequate to provide pitch angle diffusion at a rate strong enough to fill the atmospheric loss cone in the time it takes a particle to move from one end of a flux tube to the other, the maximal 'strong diffusion' rate¹⁷. It is fair to say, however, that there is more theory and experiment to be done before we confidently understand the relationship between these waves and auroral precipitation.

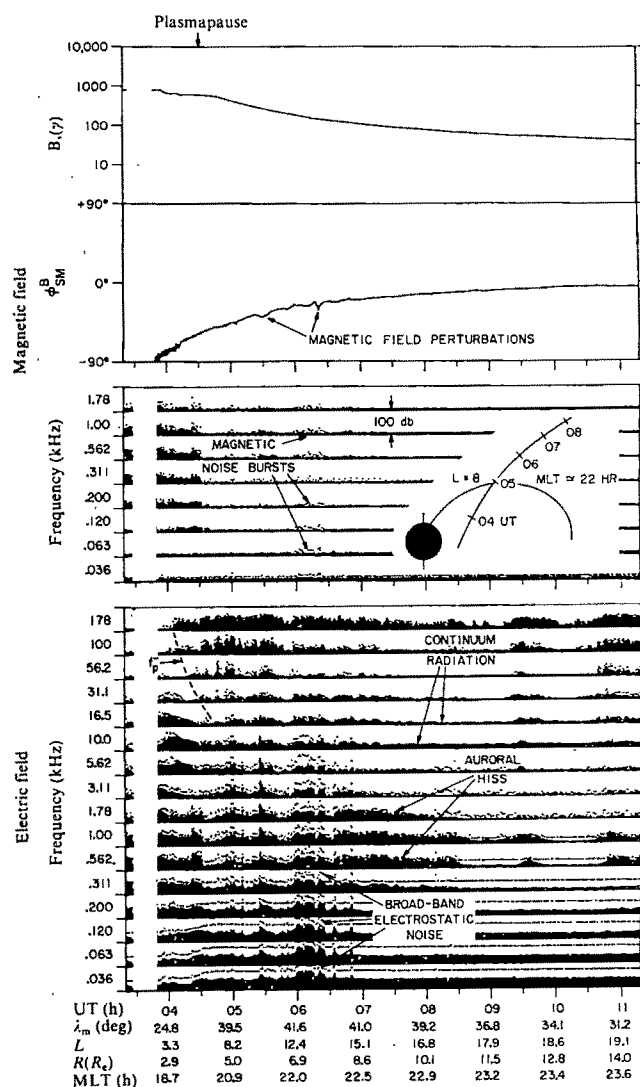
One feature of Fig. 2 deserves further comment: also shown is a noise band labelled by f_p . This plasma frequency noise is expected from cold plasma theory and it corresponds to the electron population oscillating with respect to the more massive ions. The oscillatory motion is along the magnetic field and as expected this electrostatic noise is polarised in the direction of \mathbf{B} . Another noise band is reported throughout the magnetosphere at the upper hybrid resonance frequency. Again predicted by cold plasma theory this band expands to take in the hot plasma $(n + \frac{1}{2})f_g^-$ waves in the hot electron region outside the plasmasphere. As expected the UHR noise is polarised perpendicular to \mathbf{B} (ref. 18).

Ion waves

The right hand polarised whistler mode cuts off at the electron cyclotron frequency and in cold plasma theory there is a left hand polarised wave, the ion cyclotron wave, which cuts off at the proton cyclotron frequency. Energetic protons should be able to undergo gyroresonance with this wave mode in much the same way as electrons do with the whistler mode. Somewhat parallel theories were constructed⁵ and the decay of the ring current population in the recovery phase of a geomagnetic storm (a storm is a period when enhanced convection has shrunk the plasmasphere and increased the ring current population) is consistent with pitch angle diffusion loss induced by such waves¹⁹.

The waves are expected to occur at the high frequency end of the geomagnetic pulsation band (of which more later) and one type of emission in this band, called 'pearls', correlates with the recovery phase of storms²⁰. A second type of emission, intervals of pulsations with diminishing period (IPDP) is associated with substorms and has been identified simultaneously in space but without the decreasing period feature. This probably means

Fig. 3 Imp 6 spacecraft wave electric (lower panel) and magnetic field (middle panel) data in similar format to those in Fig. 2. The inset indicates the orbit in the late evening sector. The top panel shows background magnetic field strength and ϕ_{SM} is the azimuthal angle of the field in solar magnetospheric coordinates. For our purposes note that variations in ϕ are largely due to currents parallel to \mathbf{B} . λ_m is latitude with respect to the geomagnetic equator and R is radial distance from the Earth in R_E (Earth radii). From Gurnett and Frank²². Data are for 14 August 1971, day 226.



ground stations sample signals from a wider range of sources. Waves and particles have not been definitively correlated and some evidence that ion cyclotron waves are relatively infrequent²¹ may mean the instability is finely tuned. Theories agree that just inside the plasmopause is the place most conducive to instability and, as we have already mentioned, rather unusual proton distributions are produced there by steady convection. Ring current particles with energies below ~ 10 keV are absent and there is also a cut-off at high energy leaving particles with energies in a band ~ 10 –20 keV. If such distributions have developed the upper cut-off energy has a stabilising effect on wave growth while conversely the low energy hole in the distribution is destabilising. What is crucial is the contribution particles at upper and lower cut-offs make to the plasma pressure and this makes it feasible for such distributions to be stable to the waves.

The hole in the hot proton distribution predicted and observed at low energies would seem to be a good source of free energy for plasma waves but no correlation with waves has yet been reported. The distributions inevitably occur with the high density cold plasmasphere plasma as background and it is this cold background that inhibits the ion equivalent of the $(n + \frac{1}{2})f_g^-$ electrostatic waves discussed earlier.

Electrostatic ion waves above the ion cyclotron frequency are observed but outside the plasmasphere. Speculations on their existence had been made with the discovery of the electron waves¹² but discovery has been relatively recent²². These waves with frequencies from Hz up to kHz have peak power near 10–50 Hz and are found on flux tubes which map to auroral latitudes. The peak intensity is in the range of frequencies between the ion gyrofrequency and the lower hybrid frequency $(f_g + f_g^-)^{\frac{1}{2}}$ but extends up to f_g^- . These waves have amplitudes up to 10 mV m⁻¹.

At the upper end of the frequency range these waves are closely associated with an electromagnetic whistler mode emission called auroral hiss. Gurnett and Frank²³ report the hiss as extending outside the region of intense electrostatic signals which could indicate a common source, the electromagnetic signal being freer to propagate across the field. Downgoing hiss occurs in conjunction with intense 1–10 keV electron precipitation which in turn correlates with the discrete aurora structures and connections between this and the electrostatic waves remain an interesting source for speculation.

The signals occur over a wide range of local time and on the dayside the flux tubes containing the signals probably map to the polar cusp/magnetopause regions. The signals are found in the region where the higher latitude Birkeland currents flow and strong currents can be detected by the associated deflection of the Earth's magnetic field. Such deflections are seen within the electrostatic noise region and are associated with bursts of magnetic noise which seem to be propagating in the whistler mode but at a much lower frequency (~ 50 Hz) than the auroral hiss (kHz). Figure 3, taken from an outbound pass of the Imp 6 spacecraft on the nightside of Earth shows the electrostatic noise, auroral hiss and magnetic noise bursts²².

On the nightside the flux tubes on which the electrostatic noise occurs can reasonably be taken to map into the geomagnetic tail and it is appropriate next to mention that rather similar signals are seen in the tail regions^{23,24}. Figure 4 shows Imp 8 data 30 Earth radii down the tail near the midnight meridian. The noise is present and seen in much the same frequency bands as the noise seen much closer to Earth. Once again it is found in the regions where Birkeland currents occur, the outer edge of the plasma sheet whose signature is the depression in magnetic field seen in upper panel. The electric vector is at a large angle to **B**. Whistler mode noise bursts are also seen within the electrostatic noise region and it seems that the waves in the two regions are closely connected. One significant difference is that the far weaker tail magnetic field ($\sim 5\gamma$) means the signals are between the local lower hybrid frequency and the electron gyrofrequency, the frequency range occupied at low

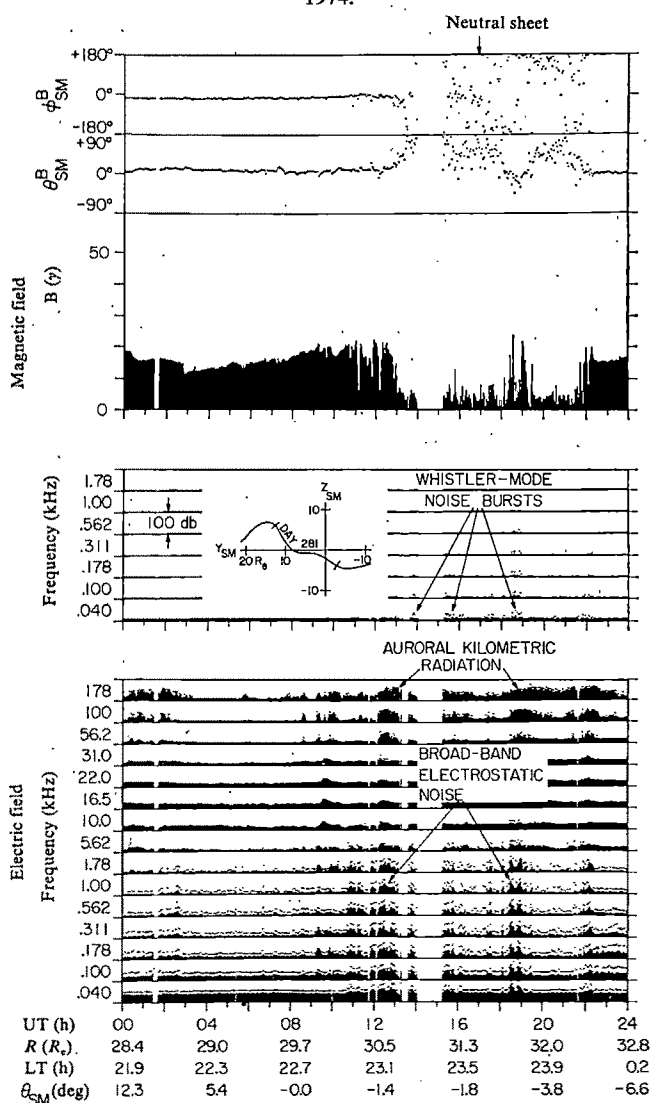
altitudes by the auroral hiss.

In the tail these signals correlate with high proton flow velocities and with auroral kilometric radiation intensity, as Fig. 4 shows. In turn the auroral kilometric radiation which we discuss later is strongly correlated with auroral activity.

Theory to describe these waves is still in the early stages of development. Several years before the observations were made Kindel and Kennel²⁵ predicted instability of ion cyclotron waves in the topside ionosphere ($\sim 1,000$ km altitude). These waves are driven by a current of cold electrons from the ionosphere. In fact the waves are seen at considerably higher altitude ($> 2,000$ km) and extend to very large radial distances.

Although a current driven source is tempting an alternative energy source is anisotropic pitch angle distributions of hot protons and there are theories predicting ion electrostatic waves in the outer magnetosphere^{26,27}. Ashour-Abdalla and Thorne²⁷ point out that these waves are strongly damped by the collisionless Landau damping process if a background cold electron population is present. If these particles are carrying a current such damping can be suppressed and the mere appearance of these waves at night and day in regions where large Birkeland currents are expected and seen argues strongly in favour of current driven instability.

Fig. 4 Imp 8 spacecraft electric and magnetic wave data in format similar to that of Figs 2 and 3 from 30 R_E down the tail. The inset indicates the orbit in a plane perpendicular to the Earth-Sun line. When **B** is depressed the spacecraft is in the plasmasheet. θ_{SM} and ϕ_{SM} are solar magnetospheric latitude and longitude. Noise is seen in conjunction with gradients in **B**. Nothing significant appears near the indicated neutral sheet crossing. From Gurnett *et al.*²⁴. Data from day 281, 8 October 1974.



Kilometric radiation

Also shown in Fig. 4 in the highest frequency channels is a signal labelled auroral kilometric radiation. These are part of Earth's radio spectrum^{2,28} which extends up to 1 MHz in frequency. As mentioned earlier these waves make the Earth at times a radio source as bright as Jupiter and the existence of these waves raises interesting theoretical problems. Gurnett² estimates that the radiated power may be as much as 10^9 W which can be placed in context by noting this is of order of 1–10% of the energy deposition rate associated with the aurora. Determination of source location by lunar occultation using the lunar orbiter RAE2 has revealed that the radiation largely originates from auroral zone field lines with most coming from altitudes between 1–2 Earth radii²⁹. There is a second source at high altitude in the cusp. The radiation correlates with auroral activity³⁰ and the flux changes by three orders of magnitude between quiet and disturbed times³¹.

These waves make a fascinating theoretical problem. There is no doubt they are in some way tapping energy available in auroral primary particles. Most theorists would agree on two points. The process is efficient ($\leq 10\%$ of auroral energy is converted into waves) and also the generation process is indirect in the sense I shall now describe. Radio waves propagate faster than light and thus it is argued cannot directly interact with a beam of auroral electrons. Rather a beam might be expected to generate electrostatic noise which then generates or is converted into the electromagnetic radio waves. Kaiser and Alexander³¹ show source frequencies are of order of the local upper hybrid and electron cyclotron frequencies and it is not unreasonable for a beam to generate electrostatic noise at such frequencies. Some theories³² then postulate conversion of the wave into a radio wave because of plasma inhomogeneity, conversion occurring when the wave frequency is near the local plasma frequency. An alternative theory³³ relies on large amplitude turbulence at the upper hybrid frequency to generate radio wave energy at twice the UHR frequency by nonlinear conversion. An ingenious idea of Palmadesso *et al.*³⁴ has a high frequency electrostatic wave generated by the beam interacting with a short wavelength low frequency ion wave to provide a high frequency long wavelength radio wave by wave-wave interaction.

All these theories have some disadvantages. On the basis of propagation cut-offs it is thought the waves are right hand polarised³⁵; the Palmadesso *et al.* theory predicts left hand polarisation. Very large amplitude electrostatic waves are required for nonlinear conversion while steep gradients are required for the most efficient conversion by inhomogeneity. There is so far little evidence of intense electrostatic noise in the appropriate frequency range though it can be argued that peculiarities of orbit coverage have left some of the expected source region relatively unexplored.

One alternative remains. It is possible for particles to see a radio wave doppler shifted to their cyclotron frequency and experience a direct gyroresonance interaction with the wave in this manner. This approach has been adopted by Melrose³⁶ who suggests the same mechanism may explain the similar radiation from Jupiter in the decametric band. Because the beam-wave interaction can be direct rather than a two-stage process it is appealing. The difficulty is that very extreme particle distributions appear to be needed if the electrons are to achieve a nett transfer of energy into the wave in the interaction.

The final story cannot then be said to be established. It will certainly involve the beams of electrons that are observed above the discrete aurora, the precise way the electrons in them are distributed in energy and pitch angle and probably also their distribution in space along with knowledge of the cold plasma distribution. It will also need to recognise the lower frequency hiss and ion waves that seem to originate in a similar vicinity. We discuss some further features of this region in the next section.

There is a second class of signal in the kilometric band as yet not definitely explained²⁸. This is the continuum radiation of much lower intensity and far more regular in character. Circumstantial evidence links this radiation with the plasma sheet electrons outside the plasmasphere and perhaps with the $3f_g/2$ electrostatic waves.

Anomalous resistivity

Anomalous resistivity remains a topic of great interest in the magnetosphere. There is circumstantial evidence of its occurrence, but much of the story waits to be developed. To understand what it is and its role, it is important to note that in a collisionless plasma one would expect the electrical conductivity along the magnetic field to be effectively infinite. The magnetospheric convection system requires currents to flow parallel to **B** as outlined earlier, though with more detailed structure expected on flux tubes that map to the neutral sheet region³⁷. Because of the high conductivity very small electric fields are required to give cold electrons a drift adequate to provide the requisite current. Once the current exceeds a threshold value, however, instability can set in. Energy from the drift motion is given up to plasma waves travelling at a similar speed to the current carrying particles. Naturally an increase in parallel electric field is then required to maintain the current. The effect of the instability is thus to increase the resistivity of the plasma.

In the presence of a turbulent wave spectrum the process is somewhat akin to the pitch angle diffusion process described earlier. Particles resonate with waves with phase speeds equal to their speed along **B** and the result of this strong interaction is that energy is fed to the wave while the particle diffuses in velocity. This process replaces the rôle of collisions in classical conductivity. If waves become strongly nonlinear the situation becomes far more complicated, wave-wave interactions may need to be considered and the radiation pressure of the waves can become important. Spatial structure is then produced by the exclusion of particles from regions where signals are most intense. Determining what stabilises a given turbulence spectrum is a taxing theoretical problem³⁸ but in the magnetosphere one test is to take an observed spectrum of waves and compute the particle scattering by the spectrum without considering the stabilisation problem. In fact given a wave spectrum regardless of the precise underlying instability one finds the effective collision frequency, ν^* , introduced by the turbulence is roughly given by

$$\nu^*/2\pi f_p \sim \epsilon_0 \langle E^2 \rangle / 2p$$

That is, the ratio of effective collision frequency to plasma frequency is of order of the ratio of the wave energy and electron pressure. Such a formula was used by Fredericks *et al.*³⁹ in a detailed study of a double sheet of current found in the polar cusp region. Wave field strengths of 90 mV m^{-1} in a frequency band stretching from below 560 Hz up to several kHz were estimated to provide an anomalous resistivity 7.9 ohm m . Using the estimate of the current density deduced from spacecraft magnetometer measurements they estimate there existed a potential drop along the magnetic field of about 2 kV, enough to provide significant auroral acceleration and precipitation.

As the effective collision rate is proportional to the square of the turbulent wave amplitude and the amplitudes reported by the Iowa group near the Earth²² and in the tail²¹ are at most a few tens of mV m^{-1} and a few mV m^{-1} respectively, resistivity due to the electrostatic turbulence emphasised in this paper is generally substantially lower. Gurnett and Frank²² suspect that in a region on appropriate flux tubes at about 1.8–4 Earth radii radial distance, as yet large unexplored, amplitudes would be higher. Some earlier reports suggest this is so⁴⁰.

Much interest centres on anomalous resistivity because of the uncertain role of parallel electric fields in acceleration of auroral

particles. In circumstances where a current of low energy particles is being disrupted, energetic particles can form a beam by running away in the parallel electric field³⁸. As mentioned earlier beams are seen in association with the low frequency noise. A recent observation⁴¹ in fact shows the noise in the presence of d.c. electric field structures suggesting a strong parallel field is present at the same time.

It is by no means a foregone conclusion that strong parallel electric fields are the prime auroral acceleration process. In fact at times the flux of energetic auroral primaries seems capable of carrying the observed Birkeland current. Causal relationships have still to be worked out in detail and another region where rapid particle acceleration is expected is the tail neutral sheet where, as briefly mentioned earlier, field lines undergo reconnection. Strong currents across the magnetic field are involved in this and one can picture the process as a conversion of magnetic energy into particle energy, but the proportions that go into thermal motions against directed motions (flows and beams) is not firmly established and probably variable. Observational information on flows in the tail is still a relatively recent development. Theories divide into quiet and turbulent current flow models. It does seem that macroscopically the flow is turbulent where the magnetic field is weak⁴² but there is a lack of any evidence of strong high frequency turbulence associated with current sheets. The only report⁴³ is of electrostatic waves of the $3f_e/2$ type with low amplitude ($\sim 300 \mu\text{V m}^{-1}$ peak intensity). This could mean beam formation could occur in the neutral sheet region without much disruption.

Hydromagnetic waves

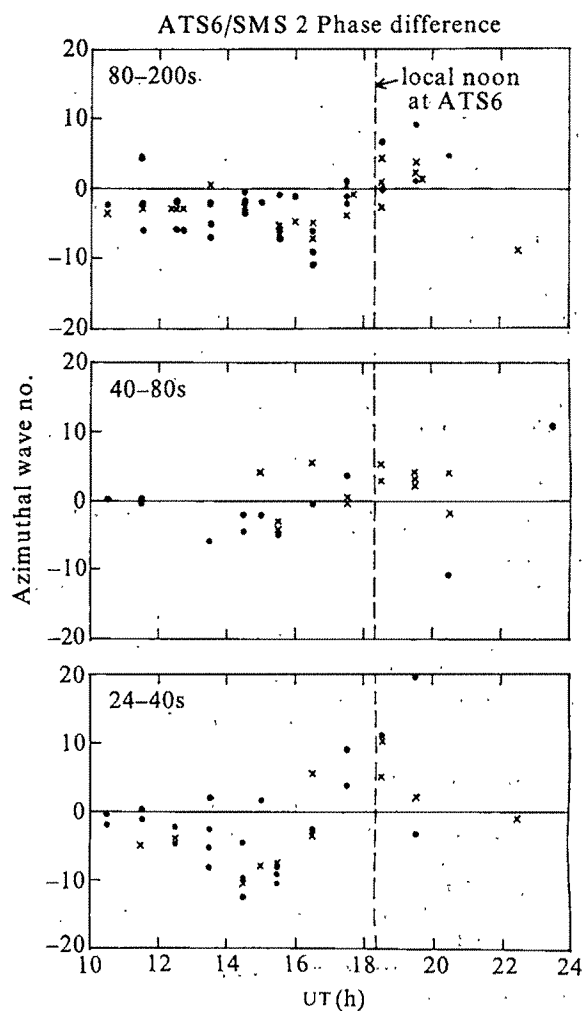
The phenomena discussed in this section are rather a contrast to those in the immediately preceding sections. Hydromagnetic waves are the lowest-frequency waves that are seen in the magnetosphere and a large number of them can be detected on the ground as geomagnetic pulsations, small fluctuations in the Earth's magnetic field. Their frequency is much below the proton cyclotron frequency and to a first approximation protons and electrons move together in the wave, the collisionless plasma behaving much like a fluid. One result of this is that waves can be described in hydrodynamic terms as well as electromagnetic. As with the background flow described earlier, the flux tube or field lines can be thought of as frozen into the wave flow and through the Maxwell stress tensor one can picture the forces which the magnetic field exerts on the plasma as a tension B^2/μ_0 along the field direction plus an isotropic pressure $B^2/2\mu_0$. A result of this approach is that one hydromagnetic wave mode can be recognised as directly analogous with waves on a stretched string. This mode, the Alfvén mode, has a magnetic perturbation perpendicular to \mathbf{B} , and thus bends the field without changing the magnetic pressure. These waves move along \mathbf{B} at the Alfvén velocity $B/(\mu_0\rho)^{1/2}$, (ρ is mass density). The equatorial Alfvén velocity is of order 10^2 – 10^3 km s^{-1} in the magnetosphere while pulsation periods range from seconds up to ten minutes. One expects wavelengths comparable to the length of the field line (from ionosphere to ionosphere) for many of the signals seen and if this is so standing wave structures form along the field lines. As neither \mathbf{B} nor ρ are uniform each field line (or more specifically field shell) has its own set of resonant frequencies. Such field line resonance signals eventually damp if only because the ionosphere does not perfectly reflect waves and the fact that the dayside ionosphere should reflect better than the nightside fits well with the observed morphology that continuous pulsations are more common by day and irregular damped pulsations more common at night⁴². However, other sinks of energy can be important including collisionless damping and also dayside and nightside energy sources do differ.

The best experimentally documented theory of dayside pulsation origin is that broadband hydromagnetic turbulence is set up near the magnetospheric boundary by the Kelvin-Helmholtz (wind-over-water) instability. The magnetosphere

then acts like a filter in that energy at a particular frequency is selectively amplified on the flux tubes that resonate at that frequency^{44,45}. The most recent evidence in favour of this source comes from magnetic field measurements made on three geosynchronous spacecraft, SMS1, SMS2, and ATS6 (ref. 46). This study showed that most signals show tailward-phase motion across the background field both in the morning and the afternoon, exactly as one would expect for waves driven by the solar wind. Some of the results are shown in Fig. 5. What is shown is the East-West angular wave number. There is a distinct tendency for the wave propagation sense to switch near local noon—best evident in the low frequency band.

Now the Hughes *et al.* study⁴⁶ is also important in showing the existence of a separate class of waves with short wavelengths across the background field. In Fig. 5 there is a gap in the data in the late afternoon (21–24 UT). Power was still present at this time of day but the signals showed little coherence between spacecraft, and thus phase difference measurement was not possible. One concludes that these signals have wavelengths shorter than the 4° longitude spacing of the geostationary spacecraft. Short wavelengths across the field are a characteristic of driftwaves, low frequency waves that occur in inhomogeneous plasmas, and these have received much attention in studies of laboratory plasma⁴⁷. Such waves are driven by extracting plasma internal energy where pressure

Fig. 5 The angular wave number perpendicular to the background magnetic field of hydromagnetic wave signals seen on two geostationary spacecraft ATS6 and SMS2. Positive wave number indicates eastward propagation and the change in sense near local midday indicates waves that are solar wind driven. The gap in the data in late local afternoon is significant and is discussed in the text. Dots and crosses distinguish polarisation differences not discussed here. From Hughes *et al.*⁴⁶.



gradients are present. There is little experimental evidence of the purely electrostatic modes that have received most attention in the laboratory context. Although measurement *in situ* of electric field is difficult at these frequencies such modes would cause particle flux oscillations and thus be detected. Electric field amplitudes of magnetic pulsation signals have been deduced in this way⁴⁸ directly confirming the standing wave structure described earlier. Back-to-back energetic proton detectors have detected wave variations on the scale of a Larmor radius⁴⁹. The particles involved in these observations are in the energetic tail of the distribution and as yet there has been no evidence that wavelengths across the field are as short as the thermal Larmor radius.

Hydromagnetic wave research can also be done on Earth relatively cheaply. Much of the field line resonance picture outlined above was developed in the light of data recorded on meridian chains of magnetometer stations on the ground. Curiously enough, measurements of East-West phase have been done only recently. Studies of waves using the British Institute of Geological Sciences magnetometer network at latitudes corresponding to inner magnetosphere flux tubes showed that their wavelengths were too long ($\sim 60^\circ$ of longitude) to be of drift wave origin while their propagation direction, preferentially eastward all day, was inconsistent with Kelvin-Helmholtz generation⁵⁰. In contrast recent high latitude measurements (Olson and Rostoker, personal communication) have shown consistency with the Kelvin-Helmholtz source like the geosynchronous spacecraft data.

Many pulsations may be driven by fluctuations in background convection and one class almost certainly is. These are the nightside 'pi 2' signals which at times can look like a text-book damped sine wave. An example of a train of such waves is shown in Fig. 6⁵¹. The upper trace was recorded near midnight in Newfoundland and shows a series of pi 2s while the lower trace was recorded in Lerwick, in the Shetlands, in the early morning. The more continuous Shetland pulsations show enhancements with the pi 2 bursts at midnight⁵¹. The midnight sector is where the most abrupt changes associated with substorms are seen. Pi 2 signals appear in close relation to the auroral intensifications, changes in convection and magnetospheric configuration. This was first pointed out ten years ago⁵² and one school now regards them as providing the best timing scheme for substorms⁵³. It seems reasonable to regard them as transients of the system associated with field lines coming to a new equilibrium with a change in convection conditions, like the twanging of telegraph wires in a gusty wind, and it would in fact be surprising if such did not occur. Their potential diagnostic use, however, is that they can appear outside the region where the most dramatic occurrences are. This is but one example of a plasma wave as a magnetospheric diagnostic. Other examples are legion and we could explore the subject much further.

Concluding remarks

This survey has not been exhaustive, but was designed to be illustrative of the type of knowledge we have. Instances of omissions are any mention of the magnetosheath, bow shock and near-Earth solar wind which closely relate to the magnetosphere and which each contain their own characteristic plasma waves known in a similar manner to those of the regions interior to the magnetosphere. We are also aware that magnetospheres other than Earth's have their own wave phenomena, with Jovian radio emissions being a prime example. Direct measurements of plasma waves within the Jovian magnetosphere have yet to be made but no doubt will be. Moving to a cosmic scale both solar and pulsar radio phenomena have been suggested to have origins similar to terrestrial radiation. In the Earth's magnetosphere we have the unparalleled advantage of direct sampling of the plasma in which the radiation originates, and of knowing the trapped plasma wave spectrum as well. Thus far these advantages have led to success-

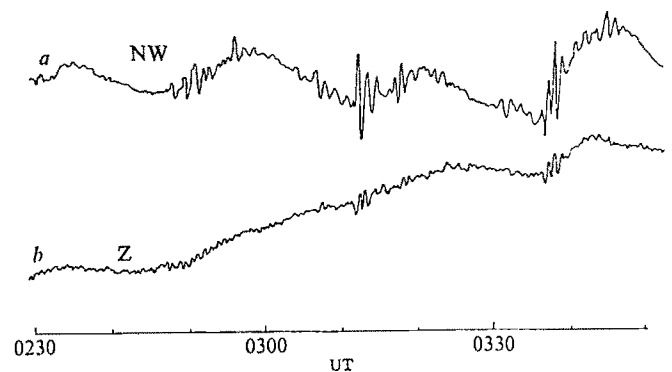


Fig. 6 Examples of nightside pulsation activity. Local time at Lerwick (b) is close to UT. St Anthony is $3\frac{1}{2}$ h behind and is near midnight. pi 2's at St Anthony (a) show as enhancements of low-level continuous activity at Lerwick. From Stuart and Booth⁵¹. Data from 21 August 1972.

ful developments of explanative collisionless plasma theory. One should also note that one often has more information about the plasma than in a laboratory plasma. Certainly the parameters a theorist wants to know (for example particle distribution in energy) are often very close to what is measured. There are, however, tantalising areas of exploration left. Often spacecraft have not flown with the right combination of instruments. International Magnetospheric Survey (IMS) activities on the ground and in space now underway should be productive. Worth a particular mention are the GEOS spacecraft of ESA and the NASA/ESA joint ISEE spacecraft projects. Both are well equipped to study waves and the latter is a multiple spacecraft project and as such the first of its kind. Happily, in the field of plasma waves alone there still remain many questions for them to answer.

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articles

Limits to the expansion of Earth, Moon, Mars and Mercury and to changes in the gravitational constant

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New estimates of the palaeoradius of the Earth for the past 400 Myr from palaeomagnetic data limit possible expansion to less than 0.8%, sufficient to exclude any current theory of Earth expansion. The lunar surface has remained static for 4,000 Myr with possible expansion limited to 0.06%, the martian surface suggests a small possible expansion of 0.6% while the surface of Mercury supports a small contraction. Observations of Mercury, together with reasonable assumptions about its internal structure, indicate that G decreases at a rate of less than $8 \times 10^{-12} \text{ yr}^{-1}$, in constant mass cosmologies, and $2.5 \times 10^{-11} \text{ yr}^{-1}$ in Dirac's multiplicative creation cosmology.

THE proposition that the Earth has expanded through geological time has received new impetus from those who claim that plate tectonics do not adequately account for many aspects of geology¹⁻³. It has been proposed that seafloor spreading exceeded subduction by 33% during the past 165 Myr, corresponding to an expansion of the Earth of over 10% since the Jurassic⁴. The possibility that the Earth or any of the planets have expanded has profound implications in cosmological theory because such expansion is predicted by models that suppose that the gravitational constant G has been decreasing with time⁵⁻⁸. It is not possible to calculate any possible changes in the Earth's radius from an examination of its surface features because these have been continually reshaped by a variety of geological processes. The only viable technique is that which uses the results of palaeomagnetic studies. In the past the number, reliability and lack of coverage of the Earth's surface of these measurements has made it difficult to make palaeoradius calculations with any accuracy. Successive analyses, however, have tended to eliminate all but the slowest ($< 2.5 \text{ mm yr}^{-1}$) expansion rates⁹. The situation has improved so much over the past decade that estimates can now, in favourable situations, be made with an accuracy of a few per cent. On the other hand, the results of the lunar exploration programme have shown that the surface of the Moon has remained relatively unchanged for nearly 4,000 Myr. Observations of the surface of Mercury and Mars by spacecraft enable constraints to be placed on the palaeoradius of these extraterrestrial bodies. Here we shall analyse palaeomagnetic data to provide constraints on the past changes in the Earth's radius and then look at the available data for the Moon, Mars and Mercury to limit possible changes that could have occurred in these bodies.

Palaeoradius of the Earth

Egyed^{10,11} first pointed out that palaeomagnetic data could be used to calculate the Earth's palaeoradius for different geological epochs. He showed¹⁰ that if two widely separated palaeomagnetic sampling sites were available for the same geological period and situated on the same palaeomeridian, the past latitude separation could be calculated from the observed palaeomagnetic inclination at each site. The ratio of the present angular separation to the past angular separation (determined by the palaeolatitude difference) was then the ratio of the past to present radius. Generally, of course, sampling sites are rarely on the same palaeomeridian and Egyed later suggested a more general triangulation method¹¹ that allows sampling sites to be arbitrarily located. Van Hiltten¹² first used this method in a much modified form and concluded that a substantial increase in the Earth's radius had occurred since the Permian. A closer analysis¹³ of Van Hiltten's method, however, has shown that his technique is incorrect and produces results that are at variance with his model. Van Andel and Hospers¹⁴ later proposed an improved triangulation technique and subsequently reviewed all estimates of the Earth's palaeoradius⁹ concluding that hypotheses involving large expansion rates^{15,16} could be rejected.

Ward^{17,18} has proposed a method which is appropriate to the spherical environment of the palaeomagnetic data. The analysis uses all data from a continental block in a single calculation. The dispersion of palaeomagnetic poles is investigated using Fisher's¹⁹ analysis of dispersion on a sphere for different values of Earth radii. That radius for which the dispersion of poles is a minimum is regarded as the best estimate of the palaeoradius for that data set. All results up to the end of 1976 and which conform with the minimum criteria for reliability set out by McElhinny²⁰ have been analysed in a new global review of palaeomagnetic data. The basic data set consists of the coordinates (λ_m, ϕ_m) of n rock units each having palaeodeclination and inclination (D_m, I_m). The Fisher¹⁹ mean position (λ_0, ϕ_0) of all rock units is calculated and the basic data are transformed to a new coordinate system with (λ_0, ϕ_0) as the pole to yield new values ($\lambda'_m, \phi'_m, D_m, I_m$) in this system. In this coordinate system, as the radius of the earth is changed (the continent keeping the same physical dimensions) so the latitude of the rock units (λ'_m) will change to some new value (λ''_m) but the longitudes (ϕ'_m) will be unchanged. For each value of radius new values λ''_m may be calculated and the resulting dispersion of the palaeomagnetic poles determined.

Table 1 lists calculations for several continents for those

Table 1 Estimates (R_a) of the Earth's palaeoradius as a fraction of its present radius

Block	Age	N	D_{\max}	K_{\max}	Data centre	Fixed centre	R_d	R_f	R_a
Africa	TR-J	18	70.7°	86.3	5.1°N, 9.1°E	5°N, 25°E	1.04	1.02	1.03
Africa	K	11	66.2°	35.6	13.2°N, 17.7°E	5°N, 25°E	(1.05)	(1.01)	(1.03)
Africa/Arabia	K	16	69.4°	29.3	13.2°N, 23.3°E	10°N, 30°E	0.99	0.99	0.99
Africa/S. America	P-C	10	64.8°	103.0	12.0°S, 1.5°E	10°S, 10°E	1.07	1.07	1.07
S. America	P-TR	10	40.9°	87.0	27.2°S, 61.7°W	15°S, 60°W	(1.21)	(1.17)	(1.19)
Africa/S. America	P-TR	13	55.0°	85.2	24.7°S, 7.5°E	10°S, 10°E	1.05	1.14	1.09
N. America	C	14	34.8°	*	45.6°N, 71.3°W	45°N, 100°W	*	*	—
N. America	P	14	37.0°	110.0	38.9°N, 100.7°W	45°N, 100°W	*	1.08	—
N. America	TR	28	38.1°	57.5	43.1°N, 89.0°W	45°N, 100°W	1.03	1.05	1.04
N. America/Greenland	J	10	45.3°	36.6	42.6°N, 88.1°W	45°N, 100°W	0.95	0.96	0.95
N. America	K	12	52.8°	65.0	44.5°N, 103.1°W	45°N, 100°W	0.98	0.98	0.98
Europe	D	24	40.1°	62.4	57.5°N, 40.1°E	55°N, 25°E	0.93	0.96	0.94
Europe	C	49	46.7°	57.5	54.6°N, 18.3°E	55°N, 25°E	0.99	0.99	0.99
Europe	P	63	41.1°	91.2	53.6°N, 29.7°E	55°N, 25°E	1.17	1.16	1.16
Europe/Asia	J	12	42.3°	33.8	46.3°N, 51.9°E	50°N, 80°E	1.00	0.95	0.98
Europe	K	14	60.8°	48.7	45.4°N, 49.5°E	55°N, 25°E	0.90	0.88	0.89
Asia (Siberia)	TR	36	32.6°	51.0	66.7°N, 100.8°E	60°N, 95°E	0.96	0.97	0.96
Asia	K	14	42.1°	42.7	58.9°N, 130.2°E	60°N, 95°E	1.03	1.03	1.03

R_a using palaeomagnetic data and Ward,^{17,18} method. R_d is the estimate using the average site location (data centre) as the pole for the coordinate system used in the calculations and R_f is the estimate using the approximate centre of the continental block as pole. N is the number of palaeomagnetic results used, D_{\max} is the largest great circle distance (in degrees) between any two palaeomagnetic locations and K_{\max} is the maximum value of Fisher's¹⁹ precision parameter for the palaeomagnetic poles. * No solution is possible.

Continental combinations use standard reconstructions^{22,23}. The values in brackets include data used in the larger set immediately following in each case. Calculations have only been listed where 10 or more locations are available.

geological periods where the palaeomagnetic results number 10 or more. One criticism of Ward's method is that the mean position of the rock units investigated is taken as the point at which strain due to curvature changes is zero. This might be far removed from the actual central point of the continent. The calculations have in each case been repeated using an approximate fixed central point in each continent. The differences between R_d (data centre) and R_f (fixed centre) calculations are negligible. So as to be unbiased, the mean, R_a , of these two values is taken as the best estimate in each case. In some instances Ward's method gives no solution because the data have been collected from too small a region of the continent. In many instances the change of the Fisher precision parameter K with radius is small and the solution for the ancient radius is not well determined. The best solutions in Table 1 are those in which there is the largest distance between the most widely separated rock units coupled with the largest number of observations. Even so the best of these determinations, probably from the Triassic–Jurassic of Africa, is only accurate to about 10% (ref. 21).

The data of Table 1 exhibit no systematic variation of radius with time from Devonian through to the present. The average radius over the past 400 Myr expressed as a function of the present radius is calculated as 1.01 ± 0.03 at the 95% confidence level taking the mean of the 14 independent estimates listed in Table 1. We can, however, improve the accuracy of the individual determinations by combining the data into larger sets, such as combining the data for Europe and Asia in post-Permian times or else reconstructing the continents into larger blocks that are known to have existed in the past. The first six entries of Table 2 involve a reconstruction of the Atlantic²² (Europe–North Amer-

ica, or Africa–South America) about which there is little dispute. Each of the six entries involves more than 25 rock units with a spread of at least 6,000 km between the most distant ones. The accuracy of each of these estimates of palaeoradius is probably about 5% at the 95% confidence level. The lower half of Table 2 gives calculations based on the Smith and Hallam²³ reconstruction of Gondwanaland and for the Triassic–Jurassic combining also with North America²². The accuracy associated with these calculations depends on one's view of the precision of the reconstruction used.

The first six entries of Table 2 provide the best overall estimates of the palaeoradius from all the palaeomagnetic data. Figures 1 and 2 illustrate the solutions for the presently largest single block (Eurasia) and for a reconstructed block (Africa–South America). Once again the results indicate no systematic change of radius with time since the Devonian. For the past 400 Myr the average palaeoradius may be calculated as 1.020 ± 0.028 (95% confidence limits). This value suggests a slight contraction of the Earth rather than an expansion. Any expansion is limited to less than 0.8% in 400 Myr. This corresponds to a maximum possible expansion rate of 0.13 mm yr^{-1} , a value sufficiently small not only to exclude the fast expansion of rates proposed by Carey^{2,15} and Hilgenberg¹⁶, but also to exclude the much slower rates proposed by Egged²⁴ (1 mm yr^{-1}) or Wesson²⁵ (0.6 mm yr^{-1}). The data are marginally consistent with the contracting Earth models of Lyttleton⁵⁰, but the phase-change hypothesis in Lyttleton's theory is inconsistent with present knowledge of the equation of state of dense matter⁵¹, and the enormous latent heat required (equivalent to a temperature drop of 10,000K) was incorrectly omitted in his thermal evolution calculations.

Table 2 R_a of the Earth's palaeoradius using large combinations of palaeomagnetic data

Block	Age	N	D_{\max}	K_{\max}	Data centre	Fixed centre	R_d	R_f	R_a
N. America/Europe	D	30	67.9°	56.3	58.6°N, 23.6°E	50°N, 10°W	1.04	1.06	1.05
N. America/Europe	C	63	82.0°	63.1	54.5°N, 5.4°E	50°N, 10°W	0.98	0.99	0.99
N. America/Europe	P	77	77.4°	89.9	57.0°N, 12.9°E	50°N, 10°W	0.95	0.98	0.97
Eurasia	TR	58	69.5°	55.1	66.5°N, 67.4°E	55°N, 60°E	1.02	1.03	1.03
Eurasia	K	28	95.9°	46.0	59.1°N, 82.2°E	50°N, 80°E	1.06	1.07	1.06
Africa/S. America	P-J	35	77.9°	62.4	10.0°S, 6.1°E	10°S, 10°E	1.03	1.02	1.02
Gondwana	P-C	16	116.9°	100.6	23.3°S, 25.5°E	15°S, 40°E	1.07	1.07	1.07
Gondwana	P-TR	23	107.6°	43.7	25.6°S, 32.0°E	15°S, 40°E	1.03	1.03	1.03
Gondwana	TR-J	35	113.8°	74.6	13.0°S, 26.8°E	15°S, 40°E	0.99	0.98	0.98
Africa/S & N. America	TR-J	43	85.5°	61.8	13.7°N, 9.5°W	0°N, 0°E	1.07	1.07	1.07
Gondwana/N. America	TR-J	56	130.7°	61.9	4.2°N, 5.7°E	10°S, 30°E	1.03	1.02	1.03

Symbols as in Table 1. Reconstructions of Bullard *et al.*²² and Smith and Hallam²³ used.

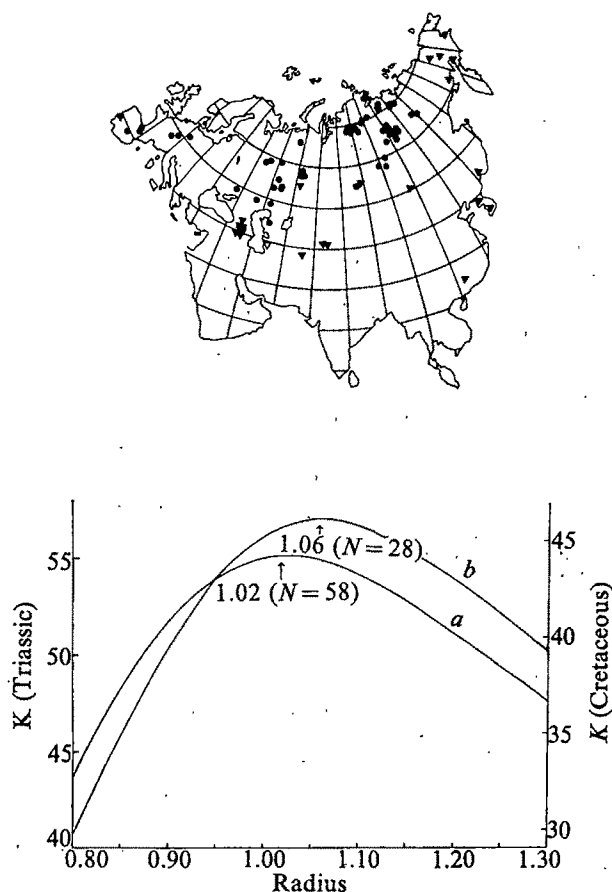


Fig. 1 Calculation of the palaeoradius for *a*, Triassic and *b*, Cretaceous palaeomagnetic data from Eurasia. The variation of the Fisher¹⁹ precision parameter *K* for the palaeomagnetic poles is shown as a function of radius (past/present) below the locality map (●, Triassic localities; ▲, Cretaceous localities).

Moon, Mars and Mercury

The surface of the Earth is not an ideal location in which to seek evidence of expansion. Although the geological record extends to 3,700 Myr, plate tectonic activity in the past 200 Myr is chiefly responsible for the presently observed surface structures. The inherent difficulties in interpreting the geological record are well known, and it is salutary to recall that the fleeting nature of the present landscape became an accepted fact less than 150 yr ago^{26,27} while the dynamic processes of recent seafloor spreading and continental movement became established only within the past 15 yr.

In contrast, the surface of the moon has remained apparently static for more than 3,000 Myr. Telescopic and orbital mapping, aided by manned lunar landings have established the essential features of the lunar surface^{28,29}. The terrain is divisible into two major areas, the maria and the highlands. The latter form an older heavily cratered surface. The origin of the large craters by meteoritic or asteroidal impact is now established. Evidence for the bombardment of the lunar surface by larger (~50 km diameter) objects is provided by the recognition of over 40 large (>220 km diameter) ringed basins, saturating the highland surface. Similar basins occur on Mercury³⁰ (for example, Caloris basin) and Mars³¹ (for example, Hellas, Argyre). Following the excavation of the basins, basaltic lavas flooded 17% of the lunar surface³². Since then, the only visible changes have been the production of younger impact craters (such as Tycho, Copernicus, Kepler) of which 15 are visible telescopically on the Earth-facing side.

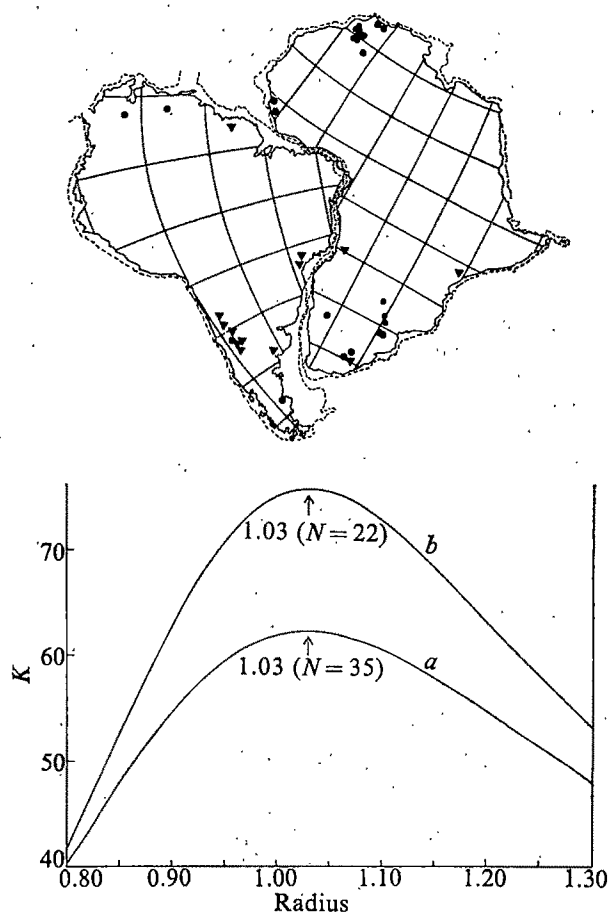
A significant achievement of the Apollo Programme was the establishment of the absolute age of these events. This knowledge, obtainable only from the returned rock samples collected by the

astronauts, constitutes a major scientific justification for the manned lunar landings. The highland crust formed before 4,400 Myr and was subjected to a massive bombardment which terminated at 3,900 Myr (refs 33–35). The basalt lavas, generated by partial melting deep (100–400 km) in the lunar interior, poured across the highland surface for at least 700 Myr, beginning at or just before the termination of the bombardment of the highland crust (3,900 Myr) and continuing to at least 3,200 Myr (ref. 29). Possibly some late flows continued to about 2,600 Myr (ref. 32). The surface has remained essentially static since 3,200 Myr. Most of the geological record preserved on the Earth postdates these events. There is no evidence of large-scale expansion or contraction³⁶ or any displacements resembling those produced by plate motions on the Earth.

Some minor features of lunar topography call for comment. In the highlands, some alteration of crater shapes has occurred. Such occasional distortions are attributable to the degradation of craters during subsequent cratering or basin-forming events³⁷. These massive events transport debris widely across the lunar surface, with extensive formation of secondary craters³⁸, and crater chains. Many effects previously attributed to volcanic or tectonic activity on the lunar surface are now explained by this mechanism^{37,38}. These basin-forming events terminated at 3,900 Myr (ref. 33). Faint lineations, referred to as the 'lunar grid'³⁹ have been detected by some workers. These may represent a faint residual trace of an early crustal cooling history. The 'grid', however, may result principally from the overlapping of outthrown debris produced by the giant basin forming collisions. In any event, it was produced before 3,900 Myr.

On the mare surfaces, wrinkle ridges³⁶, concentrated around

Fig. 2 Calculation of the palaeoradius for late Permian to early Jurassic data from Africa and South America after reconstruction of the South Atlantic according to the fit of Bullard *et al.*²² *a*, Permo-Jurassic; *b*, Triassic-Jurassic. The variation of the Fisher¹⁹ precision parameter for the palaeomagnetic poles as a function of radius (past/present) is shown below the locality map (●, Triassic-Jurassic localities; ▲, Permo-Triassic localities).



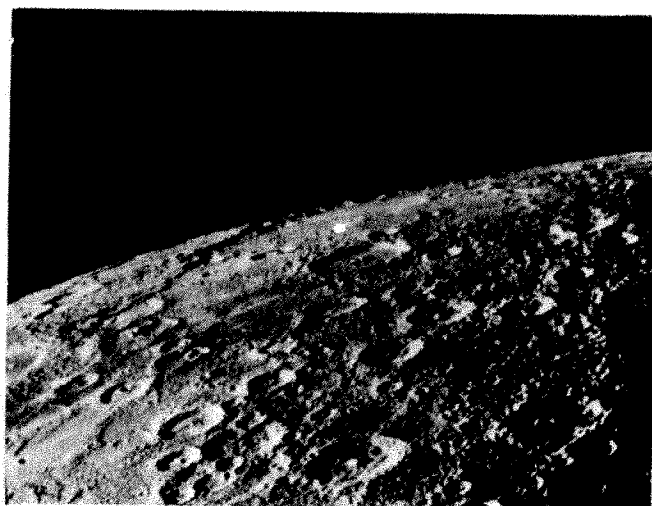


Fig. 3 Mariner 10 photograph of the northern limb of Mercury, showing a prominent east facing scarp, extending from the limb near the centre of the photograph, southwards for hundreds of kilometers. The linear dimension along the bottom of the photograph is 580 km. The picture was taken at a distance of 77,800 km from Mercury. (NASA Photograph P. 75-61-JPL-654-5-75).

the rims of the circular maria, seem to be due to localised cooling and draping of the lava sheets over pre-existing ridges. Occasional small grabens (straight rilles) in the maria are attributable to local cooling and shrinkage. Radial and concentric fractures in some basalt-filled craters²⁸ (for example, Gassendi, Humboldt) are apparently due to the same cause. The lack of present moonquake activity associated with these features is additional evidence for their antiquity. The most impressive features of the mare surface on a global scale are their smoothness (attributable to the low viscosity of the iron-rich lunar lavas) and the absence of evidence of tectonic movement since solidification.

The surface of the moon thus seems to have been frozen in its present form since about 3,200 Myr. It is possible to extrapolate this to 3,900 Myr when the last major structural events (formation of the Imbrium and Orientale ringed basins) occurred³³.

In contrast to the Moon, Mars shows evidence of slight expansion^{31,36,41}. Estimates of the chronology of the complex geological history discernible on Mars are not well established, being dependent on crater flux estimates and analogies with events on the moon^{42,43}. An early heavy bombardment produced the cratered terrain, still preserved in the southern hemisphere³¹. The next significant event seems to be the commencement of the uplift of the Tharsis region. This continent-size plateau is now about 6,000 km across and up to 10 km high. Radial fracturing of the martian crust accompanied this uplift. The next stage was the widespread production of volcanic plains, covering most of the northern hemisphere. A second stage of the Tharsis uplift now occurred, with further radial fracturing and the formation of large troughs. Finally the build-up of the large volcanic edifices (such as Olympus Mons) took place.

The dating of these events is difficult due to uncertainties in the crater flux rates. Present views^{42,43} date the old cratered terrain at about 4,000 Myr or older, from analogy with the lunar highlands.

The final uplift of the Tharsis plateau occurred about 1 Myr ago, and the large volcanoes are perhaps 200 Myr old^{42,43}. The Tharsis uplift thus seems to have occurred between about 4,000 and 1,000 Myr ago.

No major tensional features are observable on Mercury⁴³⁻⁴⁵. Extensive lobate scarps (Fig. 3) (20–500 km long and from 0.2–3 km high) provide evidence of contraction, with an upper limit of about 2 km since the formation of the crust. This effect is attributed to the contraction of a 600 km thick silicate shell around a cooling iron core⁴⁵. These scarps clearly formed later than the crust itself. From the observable crosscutting relationships, the formation of the scarps occurred close to the end of the period of major bombardment of the Mercurian surface, extending possibly a little beyond the stage^{44,45}. By analogy with the lunar highland surface, which the Mercurian landscape greatly resembles⁴⁴, the age of the heavily cratered surface is at least 4,000 Myr.

Table 3 provides estimates of the maximum change in planetary radii. For the moon, the expansion or contraction since 3,900 Myr is $< \pm 1$ km. For Mercury, a contraction of about 2 km has occurred probably before about 4,000 Myr (ref. 46). Since then, there is no evidence to support any further contraction or expansion. We therefore place limits of ± 1 km for the change in radius of Mercury over the past 3,000 to 4,000 Myr.

For Mars, the problem is more complex. The Tharsis Plateau is here assumed to be an uplifted feature due ultimately to thermal expansion^{31,36}, the case most favourable for variable G hypotheses. An alternative that it is a residual bulge following planetary contraction is less obviously reconcilable with the geomorphic evidence³¹. The maximum expansion has been estimated from thermal model calculations³⁶ to be about 19 km between 4,600 and 1,000 Myr. There seems to be no evidence for any expansion over the past 1,000 Myr. In Table 3, we give two estimates of the possible change in martian radius. Mars Model A assumes a 19 km expansion over 3,600 Myr, corresponding to the earlier martian record. Mars B assumes a possible ± 1 km change in radius during the past 1,000 Myr.

Variation of gravitational constant

Cosmologists have generally accepted the view that distant matter in the universe spherically distributed about the Earth has no noticeable effect on the Solar System. As the Universe expands and distant matter moves away from us it is supposed that there are no locally induced consequences. Exceptions to this view have been propounded by Dirac⁵, Jordan⁶, Brans and Dicke⁷, and Hoyle and Narlikar⁸. Jordan⁴⁷ later adopted a new cosmology closely paralleling that of Brans and Dicke. If the expanding Universe results in changing effects on distant matter, the consequences are a steadily decreasing gravitational constant. The Brans–Dicke theory of gravitation proposes a decrease in G of about $1 \times 10^{-11} \text{ yr}^{-1}$, for $\omega = 6$, where ω is the scalar coupling constant⁴⁸. But, recent lunar laser ranging data⁴⁹ require $\omega > 29$, and this limits the decrease in G to about $2 \times 10^{-12} \text{ yr}^{-1}$ for this theory.

In contrast, the theories of Dirac⁵² and Hoyle and Narlikar⁸ predict decreases of about $5 \times 10^{-11} \text{ yr}^{-1}$, when G is measured in atomic units. In Dirac's theory, there is also continuous creation of matter, and Dirac proposed two possibilities: additive creation and multiplicative creation⁵². In additive creation, matter is

Table 3 Palaeoradii (R_a) given as a function of present radius for the Earth, Moon, Mars and Mercury

Planet	R_a	α	Time (10^9 yr)	Constant mass	$-\delta G/\text{Gyr}^{-1}$	Multiplicative creation
Earth	1.020 ± 0.028	0.085 ± 0.02	0.4	$\leq 3 \times 10^{-10}$		$\leq 5 \times 10^{-9}$
Moon	1.0000 ± 0.0006	0.0004 ± 0.001	3.9	$\leq 5 \times 10^{-11}$		$\leq 1.5 \times 10^{-10}$
Mars A	0.9944	0.03 ± 0.01	3.6	$\leq 8 \times 10^{-11}$		—
Mars B	1.0000 ± 0.0003	0.03 ± 0.01	1.0	$\leq 1.5 \times 10^{-11}$		$\leq 4.5 \times 10^{-11}$
Mercury	1.0000 ± 0.0004	0.02 ± 0.005	3.5	$\leq 8 \times 10^{-12}$		$\leq 2.5 \times 10^{-11}$

The theoretical parameter α (see equation (3)) is listed and the corresponding maximum limits to the rate of decrease of the gravitational constant G are indicated. The models corresponding to Mars A and B are explained in the text.

created uniformly throughout the Universe and only the variation of G affects the planetary structure. In multiplicative creation, matter is created locally in proportion to the matter already present. In this case, the mass of a planet M increases at twice the rate as G decreases.

If the planet is in hydrostatic equilibrium then the pressure P at radius r within the planet satisfies

$$\frac{dP}{dr} = -\frac{G\rho(r)M(r)}{r^2} \quad (1)$$

where $M(r)$ is the mass within radius r . This can be rewritten as

$$\frac{dP}{dr^*} = -GM^{2/3}\frac{\rho(r^*)M^*(r^*)}{r^{*2}} \quad (2)$$

where $r^* \equiv r/M^{1/3}$, $M^*(r^*) = M(r)/M$. It follows that $R^* \equiv R/M^{1/3}$, where R is the radius of the planet, is a function only of $GM^{2/3}$ and the equation of state $P(\rho)$. A homologous change ($R \propto M^{1/3}$) would not be detectable in the observations we consider, since it scales all linear dimensions equally, so only variations in R^* need to be considered. In Dirac's Multiplicative Creation (DMC), $G \propto t^{-1}$ and $M \propto t^2$, where t is the elapsed atomic time, so $GM^{2/3} \propto t^{1/3} \propto G^{-1/3}$. It follows that for our considerations, DMC is exactly equivalent to a constant-mass theory in which G increases at one third of its actual rate of decrease. We can, therefore, restrict the following analysis to constant M .

The variation of R as G changes can be parameterised as

$$\frac{1}{R} \frac{dR}{dt} = -\frac{\alpha}{G} \frac{dG}{dt} \quad (3)$$

where α is to be calculated as a function of G and M . For a given $P(\rho)$ and the boundary condition $P(r=R)=0$, the solution of equation (1) for different values of G enables one to calculate α . For the particularly simple case of a polytropic gas, $P = C\rho^n$, dimensional considerations alone demonstrate that $\alpha = 1/(3n-4)$, independent of G and M . This result was applied by Hoyle and Narlikar⁵³ to the Earth, and by Crossley and Stevens⁵⁴ to Mercury, but neither application is valid as $\rho = 0$ at $P = 0$ in a polytropic gas, whereas the surface density of the terrestrial planets is finite. Because the value of α for small planets is required, it is more appropriate to derive a theory for the limit of small gravitational self-compression. This is certainly valid for the Moon, Mercury and Mars; and can be extended to include the Earth. Neglecting phase transitions and chemical layering for the moment, it is sufficient in this limit to set $P = K_0(\rho - \rho_0)/\rho_0$ in equation (1), where ρ_0 and K_0 are the zero-pressure density and incompressibility. Solution of equation (1) to lowest nonvanishing order in G is then:

$$\rho = \rho_0[1 + (\Delta\rho/\rho_0)(1 - r^2/R^2)]$$

$$\Delta\rho/\rho_0 \equiv 2\pi G\rho_0^2 R^2/3K_0 \quad (4)$$

The total mass M can be evaluated. The requirement that M be constant, as G and R change, gives

$$\alpha = \frac{2}{15} \left(\frac{\Delta\rho}{\rho_0} \right) \quad (5)$$

where $\Delta\rho$ is the density difference between planetary centre and surface caused by gravitational self-compression. A better approximation for α can be found by replacing ρ_0 by the average density $\bar{\rho}$ and K_0 by the average incompressibility \bar{K} . If the planet consists of chemical layers (each of which has constant mass as G changes) then $\Delta\rho/\bar{\rho}$ can be replaced by $\sum \Delta\rho_i/\bar{\rho}_i$, where $\Delta\rho_i$ is the density contrast across layer i of average density $\bar{\rho}_i$.

Univariant phase transitions (such as the olivine-spinel transition in the Earth and in Mars) cannot be treated this way, as the mass in each layer changes as G changes. For a homogeneous planet, the contribution to α because of a phase transition is

$$\alpha_{ph} = \frac{(\rho_1 - \rho_2)R_1(R_2^2 - R_1^2)}{2\rho_2 R^3} \quad (6)$$

in the limit of small self-gravitation, where $\rho = \rho_1$ for $0 \leq r \leq R_1$ and $\rho = \rho_2$ for $R_1 \leq r \leq R$.

For the Earth, equation (5) is a rather poor approximation and an extension of Birch analysis⁵⁵, including the olivine-spinel and spinel-postspinel transitions⁵⁶, gives $\alpha = 0.085 \pm 0.02$.

For Mars, the models of Okal and Anderson⁵⁷ (including the olivine-spinel transition) give $\alpha = 0.032$, whereas models incorporating a high pressure phase of magnetite⁵⁸ predict $\alpha \approx 0.028$. The martian moment of inertia is still uncertain⁵⁹, but $\alpha = 0.03 \pm 0.01$ encompasses all likely models.

Since Mercury provides the best constraint on the changes in R and G , the calculation for α has been done by both the simple method (equation (5)) and by the solution of equation (1) for several closely spaced values of G . For the differentiated models of Siegfried and Solomon⁶⁰ and their equations of state, the methods agree to within 20% and imply $\alpha = 0.02 \pm 0.005$. (The error is intended to indicate the likely uncertainty in the interior models of Mercury, given that the average density implies a predominantly iron composition).

For the Moon, any acceptable iron core has a negligible effect; and equation (5) applied to Ringwoods' models⁶¹ gives $\alpha = 0.004 \pm 0.001$.

The palaeoradii and these estimates for α give the upper limits to the rate of decrease of G listed in Table 3. Note that the limits for DMC are based on the limits for contraction of the planets, in accordance with our earlier discussion. To assess the validity of these limits, it is necessary to examine critically the assumption of hydrostatic equilibrium. Suppose that only an outer rigid lithosphere of thickness d violates hydrostatic equilibrium. As G decreases at constant R , the tensile stress in this lithosphere is about $3(\Delta G/G)\alpha K_0 R/d$. For $\Delta G = 0.03G$ (the upper limit implied by constant-mass Mercury), this stress exceeds 10 kbar, provided d is less than about 1,000 km (Earth, Mars), 500 km (Mercury), and 100 km (Moon). Since the lunar lithosphere is almost certainly much thicker than 100 km⁶², the allowable change in G might not affect the lunar surface. In contrast, the Mercurian mantle is only about 650 km thick⁶⁰, and the lithosphere is much thinner than this, as the presence of a large magnetic field suggests a thermally active planet with a large iron core^{63,64}.

If we exclude a fortuitous cancellation of thermal contraction and the expansion resulting from decreasing G in Mercury, then the upper limit of $8 \times 10^{-12} \text{ yr}^{-1}$ for the rate at which G decreases in a constant mass theory is realistic. This would seem to rule out the Dirac additive creation and Hoyle-Narlikar theories. The limit indicated by palaeomagnetic measurements on Earth is similar to that found from an analysis of radar observations in the inner Solar System^{65,66} and approaches the values predicted by the Dirac additive creation and Hoyle-Narlikar theories.

The upper limit for Dirac multiplicative creation of $2.5 \times 10^{-11} \text{ yr}^{-1}$ is a factor of 2 smaller than the value of $5 \times 10^{-11} \text{ yr}^{-1}$ indicated by the presently favoured value of Hubble's constant. This is a stronger constraint on the theory than other entirely different considerations⁶⁷⁻⁶⁹ have imposed.

Received 13 July; accepted 3 November 1977.

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West Antarctic ice sheet and CO₂ greenhouse effect: a threat of disaster

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If the global consumption of fossil fuels continues to grow at its present rate, atmospheric CO₂ content will double in about 50 years. Climatic models suggest that the resultant greenhouse-warming effect will be greatly magnified in high latitudes. The computed temperature rise at lat 80° S could start rapid deglaciation of West Antarctica, leading to a 5 m rise in sea level.

ATMOSPHERIC carbon dioxide traps some of the long-wave radiation emitted by the Earth's surface (principally near 15 μm wavelength), thereby tending to warm the troposphere. This so-called greenhouse effect has long been suspected^{1,2} but only recently, as the implications of a continuation of the current near-exponential growth of industrial CO₂ production have been realised, have many come to fear a disastrous climatic warming in the rather near future. In a recent report on the climatic effects of energy production, Revelle *et al.*³ conclude that industrial civilisation may soon have to decide whether or not to make the tremendous investment of capital and effort needed to change over from fossil fuels to other sources of energy. Bolin⁴, in hearings before the U.S. House of Representatives, points out that if all reserves of fossil fuel that are accessible by present techniques were burnt, thereby increasing atmospheric CO₂ content between five- and eightfold above its preindustrial level, the result would almost certainly be climatic disaster.

I contend that a major disaster—a rapid 5 m rise in sea level, caused by deglaciation of West Antarctica—may be imminent or in progress after atmospheric CO₂ content has only doubled. This concentration of CO₂ will be reached within about 50 years if fossil fuel continues to be consumed at its recent accelerating rate, or within about 200 years if consumption is held constant at today's level^{4,5}. Keeling and Bacastow⁶ believe that even if a policy of conversion to other sources of energy was started today and vigorously pursued, the global rate of consumption of fossil fuels would still double by the end of this century.

If so, the actual doubling time for atmospheric CO₂ content is likely to be nearer 50 than 200 years.

Many attempts have been made to estimate by climatic modelling the average global rise in temperature that would result from a doubling of atmosphere CO₂ content. The figures obtained have ranged from 0.7 K to 9.6 K, and Schneider⁷ has critically examined the models in an attempt to clear up the confusion created by these widely different estimates. He points out that some of the models give unrealistic results because they compute an equilibrium condition for the Earth's surface rather than for the Earth-atmosphere system as a whole. He stresses the advantages of radiative-convective models, which take into account vertical motions of the atmosphere and latent heat transport, and he compares the radiative-convective models of Rasool and Schneider⁸, who had computed an average global temperature rise of 0.8 K, with that of Manabe and Wetherald⁹ who had computed a rise of 2.3 K, later revising this to 2.9 K. He estimates that, using the most refined input of feedback mechanisms that is possible with present knowledge, globally-averaged temperatures would rise about 1.9 K. But, because some feedback mechanisms may have been improperly modelled, some (especially those involving changes in cloud cover and cloud top elevation) have been largely ignored because of our inability to do otherwise, and others perhaps remain unknown, he concludes that the best state of the art, order of magnitude estimate is that a doubling of atmospheric CO₂ content would raise average global temperature by 1.5–3 K. He believes that this estimate is as likely to be too low as too high.

More recently Augustsson and Ramanathan¹⁰, using a different modelling technique, and taking into account the previously ignored effect of bands of weak absorption by CO₂, have calculated that global temperatures would rise about 2 K. This, they note, is similar to Schneider's figure; they find this encouraging, but surprising and perhaps fortuitous because substantially different modelling techniques were used. Their one-dimensional model gives no information about differences between latitudinal zones, but the three-dimensional model of

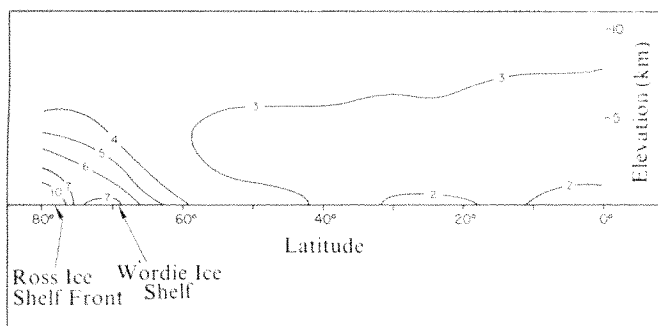


Fig. 1 Computed rise in mean atmospheric temperature, as a function of latitude and elevation, that would result from a doubling of atmospheric CO_2 content (after Manabe and Wetherald⁹ who emphasise that, because of the various simplifications of the model, the quantitative aspects of the results should not be taken too seriously).

Manabe and Wetherald⁹ shows greatly magnified warming in high latitudes caused by the lack of vertical mixing, and by the feedback effect of decreased albedo as snow and sea ice cover recede: temperatures would rise by ~ 2 K between the equator and lat 30° north or south, by 4 K at lat 60° , by 7 K at lat 70° , and by > 10 K at lat 80° (Fig. 1). Schneider⁷ agrees that this effect is likely and points out that warming in the polar regions could be crucial because of their sensitivity to changes in the energy balance.

Manabe and Wetherald⁹ emphasise that because their model is highly simplified—it has idealised global topography, fixed cloudiness, no heat transport by ocean currents and no seasonal variability—these figures for the rise in temperature should not be taken at their face value. Smagorinsky¹¹ discusses the model and points out that seasonal variability is of fundamental importance in high latitudes because it determines the extent of snow and ice cover; if this factor, and the reactions of the oceans and clouds were taken into account, a very different result might be obtained; it is even possible that global cooling would be indicated. Keeling and Bacastow⁶ conclude that until the feedback effect of the slow response of subsurface ocean waters can be correctly modelled, the regional climatic changes that are of greatest interest to mankind will be hard to predict. Nevertheless, as Bolin⁴ points out, although the model of Manabe and Wetherald has serious shortcomings it is the most advanced that has yet been developed, and to gamble with the Earth's climate by ignoring it would be highly irresponsible. In the same vein Schneider¹² sums up the dilemma facing mankind: despite the crudities and inadequacies of present techniques for modelling the climatic effects of increasing atmospheric CO_2 content and the resultant doubts about the magnitude of the warming that would actually occur, we cannot afford to let the atmosphere carry out the experiment before taking action because if the results confirm the prognosis, and we should know one way or the other by the end of the century, it will be too late to remedy the situation on account of the long residence time of CO_2 in the atmosphere (Keeling and Bacastow⁶ estimate that, if all accessible fossil fuels were burnt, restoration of pre-industrial levels of CO_2 would take at least 10,000 yr).

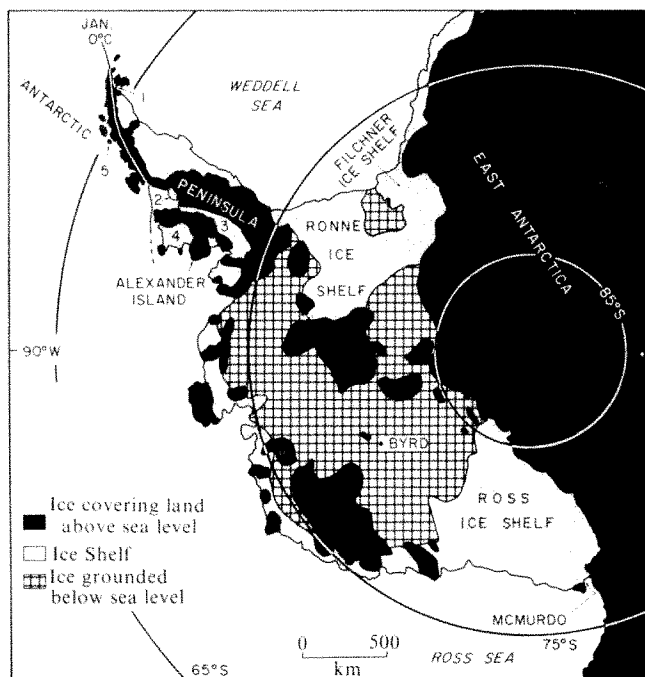
Is the CO_2 greenhouse effect detectable in recent climatic trends?

Since about 1940, temperatures over much of the Northern Hemisphere have dropped despite rising atmospheric CO_2 content. Broecker¹³ suggests that this may have lulled us into a sense of false security about the danger of increasing CO_2 levels; the cooling does not disprove or cast doubt on the CO_2 greenhouse-warming theory but is, he suspects, merely the effect of a natural cooling cycle that has overwhelmed the warming due to CO_2 , which he estimates has amounted to no more than 0.2°C for the past 30 years. He

believes that the quasi-cyclical pattern of climatic fluctuations in the recent past, which is shown by the oxygen isotope record from the Greenland Ice Sheet¹⁴, implies that this cooling will soon level out, to be followed by a period of rapid warming as the natural climatic trend is reinforced by the effects of increasing atmospheric CO_2 . In fact, the cooling since 1940 seems to have been mainly confined to middle and high latitudes in the Northern Hemisphere, and some investigators believe that the southern part of the Southern Hemisphere has warmed during the same interval. Damon and Kunen¹⁵ have studied climatic records from 67 Southern Hemisphere stations that meet certain specifications and that have records that go back to 1954 or earlier. They find that since 1943 temperatures have changed little between the equator and lat 45°S , except in Australia and New Zealand which, as other workers also point out^{16,17}, have warmed by about 1°C since the 1940s. South of lat 45°S , however, they conclude that average annual temperatures increased between the 1960–64 and 1970–74 pentads, particularly in West Antarctica where they rose about 2°C at Argentine Island (lat 65°S), McMurdo (lat 78°S), and Byrd (lat 80°S) (Fig 2). Thomas^{18,19} observation that temperatures at 10 m depth in the eastern part of the Ross Ice Shelf rose about 1°C between 1958 and 1972 confirms the warming trend in West Antarctica.

Damon and Kunen¹⁵ suggest that the climate of the Southern Hemisphere is now responding to the CO_2 greenhouse effect, whereas in the Northern Hemisphere this has recently been overwhelmed by cooling caused by man-made particulate pollution and also, possibly, from greater volcanic activity. Budd¹⁹, however, while noting the recent warming in West Antarctica, finds no clear evidence that Antarctica as a whole either warmed or cooled between 1958 and 1972; furthermore, at the South Pole 1976 was the coldest year since records began there in 1957²⁰. So far, apparently, despite recent regional warming over Australasia and West Antarctica, there is no unequivocal evidence for a global rise in temperature caused by increasing atmospheric CO_2 content. If Broecker's estimate is correct that average global CO_2 -caused warming would have amounted to no more than a fifth of a degree in the past 30 years, this is perhaps not surprising.

Fig. 2 West Antarctica, showing ice shelves, ice grounded below sea level, ice covering land above sea level, and position of the 0°C January isotherm in the Antarctic Peninsula (based on information up to the year 1962)²⁵. 1, Prince Gustav Channel; 2, Wordie Ice Shelf; 3, George VI Sound; 4, Wilkins Sound; 5, Argentine Island.



First disastrous consequence of rising CO₂ levels

Those who are most aware of the climatic dangers of increasing atmospheric CO₂ do not seem to view deglaciation in Antarctica as an immediate threat. For example, Schneider and Dickinson²¹, and Bolin⁴, believe that the Antarctic Ice Sheet will respond to climatic warming very slowly, during millennia, while Rotty and Budyko^{22,23} are more concerned with the shrinkage and eventual disappearance of the Arctic sea ice. Revelle *et al.*³ conclude that our present understanding of the Antarctic Ice Sheet is insufficient for us to forecast how it would be affected by global warming of several degrees. They believe that the direct effect of the warming would be minimal, but suggest that increased snowfall might eventually thicken the ice sheet enough to cause surging, perhaps resulting in deglaciation of West Antarctica. But, not only would effective thickening of the ice sheet require a long interval of increased snowfall, but also it is doubtful that a thickening ice sheet would surge. I believe that Revelle *et al.*³ underestimate the direct effect of moderate Antarctic warming, which would be likely to cause deglaciation of West Antarctica long before any significant thickening of the ice sheet could occur.

If present trends in fossil fuel consumption continue, and if the greenhouse warming effect of the resultant increasing atmospheric CO₂ is as great as the most advanced current models suggest, a critical level of warmth will have been passed in high southern latitudes 50 years from now, and deglaciation of West Antarctica will be imminent or in progress. Deglaciation would probably be rapid once it had started, and when complete would have led to a rise in sea level of about 5 m along most coasts. The reasons for this ominous situation lie in the unique characteristics of the West Antarctic ice sheet.

West Antarctic ice sheet's vulnerability to climatic warming

The Antarctic Ice Sheet consists of two unequal parts, with different histories and characteristics: the vast, long-established, mainly land-based ice sheet in East Antarctica, and the younger, much smaller marine ice sheet²⁴, grounded as much as 2,500 m below sea level in West Antarctica (Figs 2 and 3). Melting of the East Antarctic ice sheet would raise sea level by about 50 m, whereas melting of the West Antarctic ice sheet, much of which is only displacing ocean water, would raise sea level by no more than about 5 m on average²⁶ (Clark & Lingle²⁶ show that the sea level change would not be globally uniform).

When the Antarctic Ice Sheet formed while temperatures dropped during the late Cretaceous, there was an interval, during which temperatures dropped further, between the emplacement of the East Antarctic ice sheet in the late Miocene²⁷ and of the West Antarctic ice sheet, probably during the latest Miocene or earliest Pliocene²⁸. This was because the land-based East Antarctic ice sheet could form as a temperate glacier, whereas the marine West Antarctic ice sheet had to consist of cold ice from the start²⁵. (The thermal requirements of the West Antarctic ice sheet are discussed in more detail later.) Thus, in order to survive, the West Antarctic ice sheet needs colder summers—perhaps as much as 10 °C colder—than does the East Antarctic ice sheet, and is, therefore, more vulnerable to a rise in temperature. During sustained climatic warming, the Miocene–Pliocene sequence of glacial buildup would be reversed, and the West Antarctic ice sheet would be eliminated before the East Antarctic ice sheet was greatly affected. Fortunately, serious depletion of the East Antarctic ice sheet is a distant threat because, being land-based, it could retain a positive mass balance after climatic warming had converted it from a cold to a temperate glacier at low elevations. Even if further warming gave it a severely negative mass balance, it would waste away only slowly over millennia, by *in situ* melting, as the Laurentide Ice Sheet did 14–8,000 yr ago²⁹. The resultant rise

in sea level would cause considerable inconvenience, but would be gradual enough to allow coastal communities to adjust. This is apparently the type of deglaciation that Schneider and Dickinson²¹ and Bolin⁴ had in mind when they considered the effects of climatic warming on polar ice sheets; unfortunately, however, the West Antarctic ice sheet would be unlikely to shrink in this manner.

In contrast to the land-based East Antarctic ice sheet, the marine ice sheet in West Antarctica can exist only so long as its grounded portion is buttressed by fringing ice shelves; in particular, by the Ross and Filchner–Ronne ice shelves (Fig. 2). Thus any environmental change that diminished or destroyed these ice shelves would also diminish or destroy the ice grounded below sea level; as the ice shelf fronts receded southward, their grounding lines would also recede and the grounded ice sheet would shrink and thin. Eventually, after all ice shelves had disappeared, ice cover would be confined to areas above sea level, and glaciers would terminate in ice cliffs between high and low water levels^{25,30–32}.

Ice shelves are vulnerable to both oceanic and atmospheric warming. They will melt at the base if the water they are in contact with is above its freezing point; as Robin³¹ notes, they are absent from the coasts of the Antarctic Peninsula only where sea temperatures rise above –1.5 °C during the warmest month. (For water with 35‰ salinity, freezing point is about –1.9 °C at the surface and about –2.2 °C at 500 m depth³³). Melting will accelerate if a positive temperature gradient develops between the base and the surface; this will happen only if the ice shelf becomes temperate as rising air temperatures produce enough meltwater to percolate downwards and destroy the previous winter's cold wave. In fact temperate ice shelves do not seem to exist in nature, except possibly as short-lived features during climatic warming; Robin and Adie³¹ observe that all known ice shelves are cold, that is, below the pressure melting point at depth, and that their northern limit on the west coast of the Antarctic Peninsula lies a short distance south of the northernmost land glaciers that are cold at sea level. They conclude that a climatic warming above a critical level would remove all ice shelves and, consequently, all ice grounded below sea level, resulting in the deglaciation of most of West Antarctica.

The observations of Robin and Adie imply that where summers are warm enough to destroy the previous winter's cold wave in a glacier at sea level, ice shelves will be absent. In the Antarctic Peninsula the 0 °C isotherm for the warmest month (January), which trends south-west from the tip of the peninsula towards Alexander Island³⁵ (Fig. 2), is almost parallel to, and a short distance outside, the northern limit of ice shelves. Other aspects of the summer climate besides air temperature, particularly the duration and intensity of solar radiation, must also be involved in determining the northern limit of cold ice at sea level, and thus of the ice shelves, but the 0 °C isotherm for midsummer air temperature seems at least as realistic a climatic boundary for ice shelves as does, for example, the 10 °C midsummer isotherm that approximately marks the northern limit of trees³⁶. Most ice shelves, however, terminate considerably south of the 0 °C midsummer isotherm. Around the coasts of East Antarctica they are limited by the positions of the outermost lateral anchor points³⁷, but in West Antarctica neither high temperatures nor lack of anchor points prevent the northward expansion of the Ross and Filchner–Ronne ice shelves. The frontal positions of these large ice shelves depend mainly on the position of the grounding line of the West Antarctic ice sheet, and this is determined by water depth and, to a smaller extent, by snow accumulation rates.

Average midsummer air temperatures at the fronts of both the Ross and Filchner–Ronne ice shelves are now about –4 °C to –5 °C³⁵. Thus the deglaciation of West Antarctica that Robin and Adie³¹ foresaw would result from sufficient climatic warming would be unlikely to start until air temperatures had risen about 5 °C, bringing summer temperatures

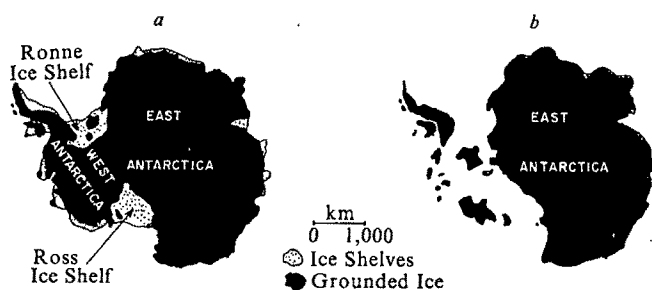


Fig. 3 *a*, Antarctic ice cover today, and *b*, after a 5–10 °C warming.

over the Ross and Filchner–Ronne ice shelves up to those now prevailing in the northwestern Antarctic Peninsula. Once this level of comparative warmth had been reached, deglaciation would probably be rapid, perhaps catastrophically so, because even a small additional rise in temperature would affect a large expanse of gently sloping ice shelf. As Hughes³² graphically describes it, “a relatively minor climatic fluctuation along the ice shelf calving barrier can unleash dynamic processes independent of climate that cause calving bays to remorselessly carve out the living heart of a marine ice sheet.” An example of deglaciation by the calving bay mechanism was the extremely rapid disintegration of the central portion of the Laurentide Ice Sheet, centred over the present Hudson Bay, sometime between 8,000 and 7,500 BP, after thousands of years of slow recession on land³⁸.

The Ross and Filchner–Ronne ice shelves would probably be on the verge of receding southward if summer temperatures rose 5 °C at lat 80° S. The climatic model of Manabe and Wetherald⁹, for which year-round climatic uniformity is assumed, suggests a warming of more than 10 °C at lat 80° S after atmospheric CO₂ content has doubled; this concentration will perhaps be reached within 50 years. If this model is even approximately correct, rather drastic deglaciation should then be in progress in West Antarctica.

One warning sign that a dangerous warming is beginning in Antarctica will be the breakup of ice shelves in the Antarctic Peninsula just south of the recent January 0 °C isotherm; the ice shelf in Prince Gustav Channel on the east side of the peninsula, and the Wordie Ice Shelf, the ice shelf in George VI Sound, and the ice shelf in Wilkins Sound on the west side (Fig. 2). There is some evidence, not yet conclusive, that such a southward recession of the ice shelf limit in the Antarctic Peninsula has already begun; a comparison of LANDSAT imagery with earlier surveys has shown that between 1966 and 1974 nearly 600 km² or about one quarter of the Wordie Ice Shelf broke away, and the ice shelf in George VI Sound receded³⁹.

Fall in sea level by glacial growth

High latitude warming might well bring increased snowfall to Antarctica and perhaps to parts of Greenland also, as Revelle *et al.*³ suggest. This is far from certain, however, because although the warmer air masses could hold more moisture, cyclonic disturbances might decrease in number and intensity if, as present models indicate, the latitudinal temperature gradient decreased. To what extent deglaciation in West Antarctica would be offset by glacial buildup elsewhere is hard to estimate. Robin and Adie³¹ calculate that doubling the net annual accumulation rate on the East Antarctic ice sheet, which already reaches the coast, would increase its volume by no more than 10%. This, they estimate, would eventually result in a 4 m drop in sea level; however, they also conclude that the East Antarctic ice sheet would take several thousand years to reach its new equilibrium volume. Thus, if temperatures rose no further, warmth-induced deglaciation of West Antarctica might eventually be partially or even wholly offset

by thickening of the surviving ice sheets. It would be unwise, however, to rely on this buildup being rapid enough to avert a disastrous rise in sea level, and in any case, if temperatures continued to rise because of increasing atmospheric CO₂ content, glacial growth in East Antarctica would eventually cease.

Previous destruction of the West Antarctic ice sheet

Considerable evidence from the Southern Hemisphere suggests that the warmest part of the last interglacial (Sangamon–Eem) was warmer than the present interglacial has been so far; for instance, subantarctic seas were then warmer than they have been since (J. D. Hays, personal communication), and in Southern Chile chemical weathering was unusually intense⁴⁰. This warm interval—substage 5e, according to the sequence established by Emiliani⁴¹—was centred 120–125,000 yr ago⁴². If the West Antarctic ice sheet was absent at that time, the hypothesis that a rather moderate rise in temperature would destroy it would be strengthened.

I earlier suggested that the high sea level of the last interglacial (probably about +6 m) resulted mainly from deglaciation of West Antarctica when temperatures there rose too high for the survival of ice shelves²⁸. Deglaciation of West Antarctica alone would add a layer of water about 5 m deep over the area of the present world ocean. Later, Emiliani suggested that the high sea level resulted from deglaciation of Greenland, with minimal contribution from Antarctica, but this is highly unlikely because the marine West Antarctic ice sheet is much more vulnerable to climatic warming than is the land-based Greenland ice sheet.^{43,44}

A rise in eustatic sea level is not necessarily a glacio–eustatic rise, so that the high sea level of the last interglacial by no means proves that less ice was then present. Supporting evidence for the hypothesis came later when oxygen isotopic analyses of Core V 28-238 from the equatorial Pacific showed that the mass of the oceans during substage 5e was greater than it is today⁴². The isotopic difference between the Holocene and substage 5e was about what might be expected from melting of the West Antarctic ice sheet during substage 5e. Shackleton (personal communication) now believes that because planktic and not benthic foraminifera were analysed for substage 5e in Core V28-238, the isotopic composition may include a temperature effect. Thus, although the oceans did contain more water during substage 5e than they do now, just how much more is uncertain. Measurements being made on other deep sea cores should give more accurate figures in the near future.

Possible disintegration of West Antarctic ice sheet from non-climatic causes

Some workers^{45–48} believe that the West Antarctic ice sheet shrank at the end of the last glaciation when its grounding line receded as the result of the ~120 m eustatic rise in sea level. Others^{50,51}, however, believe that changes in the extent and thickness of the West Antarctic ice sheet since the last glacial age have been minor. In any case, with the larger Northern Hemisphere ice sheets gone, no further major shrinkage of the West Antarctic ice sheet as the result of rising sea level will occur in the near future.

Thomas and Weertman^{52,53} believe that the present West Antarctic ice sheet is inherently unstable and that, if it disintegrates, it will do so through mechanisms unconnected with climate. Hughes⁵⁴ agrees that such internal instability mechanisms are possible and could result in surges, but he also stresses the vulnerability of the ice sheet to climatic change. Weertman⁵³ suggests that the presence of ice streams draining the ice sheet is a premonition of a large-scale surge, the start of a process that may cause the West Antarctic ice sheet to discharge one third to one half of its volume into the oceans over, say, 100 years. The only occasion, however, during the last half

million years or so that the oxygen isotopic composition of the oceans implies the presence of less land ice than today is during substage 5e (ref. 42); that is, during an interval that was warmer than the Holocene. This strongly suggests cause and effect: the West Antarctic ice sheet was absent because of the high temperature. There is no evidence that the ice sheet has ever been seriously depleted by surges in the past and therefore, I believe, there is no reason to suppose that it will be in the future. Weertman may be right for the wrong reasons; the ice sheet is likely to disintegrate in the rather near future, but because of man-made climatic warming, not through surges resulting from internal instability mechanisms.

Conclusions

The present West Antarctic ice sheet is reasonably secure against all instability mechanisms except for climatic warming above a critical and—for the Pleistocene—exceptional level. In the natural course of events, this level of warmth might be reached about once in half a million years, perhaps as a consequence of an unusual combination of the astronomic factors that seem to be responsible for the timing of the major glacial-interglacial climatic changes⁵⁵. Furthermore, because the present interglacial has apparently passed its natural peak of warmth—this was probably reached about 9,400 yr ago in the Southern Hemisphere⁵⁵—deglaciation of West Antarctica would not occur in the foreseeable future without Man's injection of massive amounts of industrial CO₂ into the atmosphere.

If the recent growth rate of fossil fuel consumption continues, atmospheric CO₂ content is expected to double in about 50 yr. Present models of the climatic effects of this doubling compute a rise in temperature that could cause rapid deglaciation of West Antarctica, leading to a 5 m rise in sea level. Although the models are known to be crude and over-simplified, so that the climatic changes that will actually occur will no doubt differ considerably from their estimates, there is, at present, no way of knowing whether the models err on the optimistic or pessimistic side.

If the CO₂ greenhouse effect is magnified in high latitudes, as now seems likely, deglaciation of West Antarctica would probably be the first disastrous result of continued fossil fuel consumption. A disquieting thought is that if the present highly simplified climatic models are even approximately correct, this deglaciation may be part of the price that must be paid in order to buy enough time for industrial civilisation to make the changeover from fossil fuels to other sources of energy. If so, major dislocations in coastal cities, and submergence of low-lying areas such as much of Florida and the Netherlands, lies ahead. More sophisticated climatic modelling may show that the outlook is less alarming than this, but on the other hand, it may show that the situation is even more threatening. The urgent need for this sophisticated modelling is evident.

One of the warning signs that a dangerous warming trend is under way in Antarctica will be the breakup of ice shelves

on both coasts of the Antarctic Peninsula, starting with the northernmost and extending gradually southward. These ice shelves should be regularly monitored by LANDSAT imagery.

I thank I. M. Whillans for helpful comments.

Received 1 August; accepted 3 November 1977.

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Can a myosin molecule bind to two actin filaments?

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It is suggested that in striated muscles the two heads of one myosin molecule are able to interact with different actin filaments. This would provide a simple explanation for the appearance and arrangement of cross-bridges in insect flight muscle in rigor.

THE myosin molecule has two globular heads attached at one end of a fibrous tail¹. During muscle contraction the heads are thought to bind to actin filaments and produce tension by tilting², but the way in which this is shared by the two heads is not known. It is widely supposed that the two heads of one myosin molecule interact with neighbouring subunits in the

Table 1 Retention of phosphate by hydrolytic precipitation products of aluminium collected from systems aged for 40 d in the absence and in the presence of citric acid

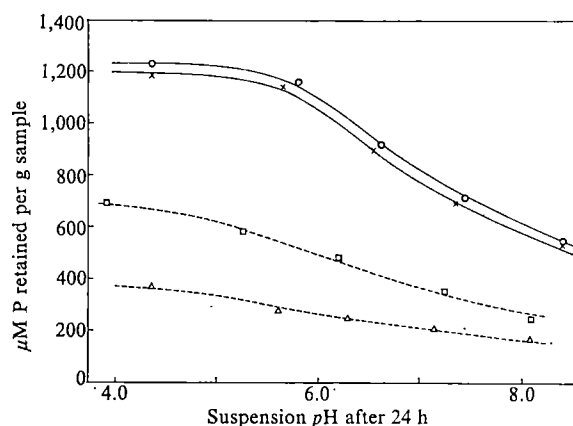
Suspension pH at equilibrium	$\mu\text{M P}$ retained per g sample		
	Citric acid concentration (M)		
	0	10^{-6}	10^{-4}
4.0	370	745	1,205
5.0	330	685	1,180
6.0	270	560	1,060
7.0	210	435	775
8.0	165	335	575

The preparation of the hydrolytic precipitation products of aluminium starting from an initial aluminium concentration of 1.10×10^{-3} M and the OH/Al molar ratio of 3.0 in the absence and in the presence of 10^{-6} M and 10^{-4} M citric acid has been described elsewhere⁹. Retention of phosphate was determined by shaking 50 mg of precipitation products in 50 ml of 0.1 M KCl solution containing 1.0×10^{-5} M KH_2PO_4 for 24 h at 25 °C. The data are deduced from a plot of $\mu\text{M P}$ retained per g sample against pH of the equilibrium suspension.

aluminium ions in aqueous solution^{10,11} and as a result of the occupation of coordination sites of aluminium by citrate during the formation of the precipitated products, distortion in the arrangement of hexagonal ring units normally found in crystalline aluminium hydroxides is bound to occur. The distortion as shown by X-ray diffraction analyses and electron microscopic observations was sufficient to impart a rough surface to the precipitation products and to cause the products formed in the presence of 10^{-6} – 10^{-4} M citric acid to be noncrystalline to X rays⁹. The mechanisms of adsorption of phosphate on hydrated aluminium oxides, on the basis of existing information¹², involve either the displacement of H_2O at positive sites or OH at negative sites. The formation of these phosphate retention sites on the surfaces of hydrolytic reaction products of aluminium would thus be promoted by the structural distortion effect of citric acid during the hydrolytic precipitation process. Moreover, the noncrystalline nature of the products enhances their specific surface (Table 2). This increase in specific surface of the products coupled with their noncrystalline nature and thus reactive sites for phosphate retention would apparently, at the low concentrations of citric acid, more than compensate the blocking of retention sites by citric acid and explains why the retention of phosphate by the products precipitated in the presence of citric acid is more extensive than in its absence.

The capacity of the noncrystalline solid phase products precipitated in the presence of 10^{-4} M citric acid to retain phosphate is not substantially diminished by an ageing period of 40 d (Fig. 1). On the other hand, the uptake of phosphate by the corresponding products formed in the absence of citric acid is considerably reduced after 40 d, for example, from 675 to 370 $\mu\text{M P}$ per g at pH 4.0 or from 270 to 165 $\mu\text{M P}$ per g at pH 8.0 (Fig. 1). Noncrystalline

aluminium hydroxides precipitated in the absence of interfering ions are very unstable and revert rapidly to the crystalline state¹⁴. On reversion to their crystalline state the specific surface of the aluminium hydroxides is considerably reduced (Table 2) with a concomitant decrease in the active sites for phosphate retention. In the presence of citric acid, the occupation of coordination sites by citrate would retard the reversion of the precipitation products to the crystalline state as that would require the removal of citrate from within the structure. The maintenance of the noncrystalline state with an accompanying high specific surface (Table 2) by citric acid would thus help to stabilise the high phosphate retention capacities of noncrystalline aluminium hydroxides.

**Fig. 1** Retention of phosphate by hydrolytic reaction products of aluminium formed in systems at the initial aluminium concentration of 1.10×10^{-3} M and OH/Al molar ratio of 3.0 in the absence (dashed line) and in the presence of 10^{-4} M citric acid (solid line) as a function of pH and time. Phosphate retention was determined as outlined in Table 1. \circ and \square , after 1 d; \times and \triangle , after 40 d.

Under natural environments, absolute elimination of low molecular weight biochemicals, such as citric acid, is seldom achieved and it is more realistic to believe that the hydrolytic reaction products of aluminium in soils and aquatic sediments are generally formed in the presence of minute but measurable quantities of biochemical compounds. Previous investigations^{5–8} have only reported the tendency of these biochemical compounds to reduce anion uptake by hydrated aluminium oxides through competition with the anions for adsorption sites. The present study provides direct evidence that they also enhance the retention of anions such as phosphate by the hydrolytic reaction products of aluminium through their promoting effects on the formation of noncrystalline aluminium hydroxides. In nature, therefore, the low molecular weight organic compounds have a dual role of hindering and promoting anion retention by the hydrated aluminium oxides depending on the mechanisms of their interfering reactions. This finding should be of fundamental importance not only in understanding the basic colloidal chemistry of aluminium but also in the transport and fate of nutrient and pollutant anions in terrestrial and aquatic ecosystems.

This work was supported by a grant from the NRC of Canada.

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Received 15 August; accepted 14 November 1977.

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Table 2 Specific surface of the hydrolytic precipitation products of aluminium in the absence and in the presence of citric acid, after 1 d and 40 d

Initial citric acid concentration (M)	Specific surface ($\text{m}^2 \text{g}^{-1}$)	
	Ageing period (d)	
	1	40
0	109.0	18.9
10^{-6}	136.6	117.1
10^{-4}	311.5	295.1

Specific surface was determined by retention of ethylene glycol monoethyl ether¹³.

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Limited response of the K–Ar system to the Nordlinger Ries giant meteorite impact

For the proper interpretation of radiometric rock ages it is important to know which processes are able to reset radioactive clocks. In particular, the resetting by intense shock effects is significant in lunar chronology because most lunar highland rocks are impact breccias produced in the course of multiple large meteorite impacts. For the important K–Ar scheme, we have dated variously shocked samples from ejecta and from a drill core through one of the best documented terrestrial meteorite craters the Nordlinger Ries (Southern Germany)¹. From K–Ar and fission track dating of thoroughly melted impact glasses the 24 km diameter Ries crater was found to have formed 14.7 ± 0.7 Myr ago by meteorite impact into the variscian bedrock (for summary of ages see ref. 2). The modern version of K–Ar dating (^{39}Ar – ^{40}Ar technique) can reveal quantitative information about partial losses of radiogenic argon (fractional resetting) in addition to yielding ages eventually not affected by these gas losses^{3,4}. We have applied this dating technique to mineral separates (hornblende, biotite, chlorite) from ejecta, fall-back breccias and underlying bedrock of the Ries Crater, including drill core samples taken at depths up to 1,201 m (ref. 1). The samples exhibit various degrees of mechanical strain (from ~ 10 kbar up to > 400 kbar shock pressure). Apart from the thoroughly molten glasses which reproduced the impact age of ~ 14.7 Myr, we found that all other rocks involved in the impact irrespective of the degree of shock metamorphism and within the limits of error, yield the same ^{39}Ar – ^{40}Ar age of 313 ± 3 Myr, probably the age of the bedrock. Apparently, rocks exposed to the very intense shock effects frequent on the Moon give reliable K–Ar ages when measured as K–Ar plateau ages. Age resetting, if present, probably results from heating associated with such impacts. In the case of the Ries, we found from the diffusion characteristics of argon that the post-shock temperature of the crystalline fragments within the suevite layer has not exceeded 600 °C.

Mineral separates were used to study the effect of shock on different minerals. We separated hornblende, biotite, and chlorite from the low grade metamorphic amphibolites and gneisses which occur as crystalline fragments imbedded in the suevite fall-back breccia above the crater bottom at 602 m depth, and as massive crystalline rocks below the crater bottom as well as in the crystalline wall of the crater (sample Meyer's Keller)¹.

The crystalline rocks from the drill core are weakly shocked and show impact features, (like, for example, planar elements) typical of shock pressures between 10 and 200 kbar (stage '0' and '1' of shock metamorphism)^{5–7}. The crater wall amphibolite (Meyer's Keller) and the gneiss from ejected suevite sampled at Otting display shock effects of stage 2 (~ 300 –450 kbar). All samples dated are listed in Table 1, including a melted impact glass and a moldavite that is, a tektite produced in the Ries impact event.

The experimental procedures applied in the ^{39}Ar – ^{40}Ar analysis are basically the same as those applied by us in dating lunar and meteoritic samples⁸. The samples obtained a fast neutron irradiation (FR2-reactor, Karlsruhe) to convert a part of the ^{39}K to the 269 yr- ^{39}Ar as a measure of the K in the sample.

Afterwards, the samples were degassed by stepwise heating from 400 to 1,500 °C (6–12 steps) and the Ar isotopes (Ar^{36} – 40) were measured by mass spectrometry. The ^{40}Ar to ^{39}Ar ratios obtained for each temperature fraction were converted into K–Ar ages by means of a muscovite monitor (Bern M4, $t = 18.0 \pm 0.2$ Myr) (ref. 9) irradiated together with the sample.

Two typical age spectra are shown in Fig. 1. In the absence of Ar-diffusion losses, an age plateau is obtained throughout essentially the whole range of degassing temperatures (Fig. 1a, hornblende from the crater bottom), whereas with the Ar-losses the geologically significant age plateau starts at intermediate temperatures and the low temperature fractions have reduced apparent K–Ar ages (Fig. 1b) ejecta from Otting.

A summary of results is given in Table 1. The character of the age spectra can be visualised from the error of the plateau age, the plateau range, and the percentage of gas loss. With the exception of the impact glass and the moldavite, both of which gave the impact age, 14.7 Myr, all samples yield plateau ages of approximately 313 Myr. The samples included depths > 700 m where the effect of the impact is minimal⁵. The average age 313 ± 3 Myr is probably the age of the basement rock. The age obtained represents the first direct dating of the pre-Ries basement rock.

The uniformity of plateau ages from all depths (above and below the crater bottom) suggests that the impact process involving enormous amounts of energy¹⁰ did not effectively disturb the K–Ar system of hornblende or biotite. This is true for ejected material as well as for crystalline inclusions in both high and low temperature suevite, and agrees with the experimental results of Davis¹¹ who demonstrated in laboratory experiments (65–270 kbar) that shock pressure alone has little influence on the K–Ar system.

For natural impacts, fractional age reductions have been observed at shock pressures above 200 kbar for anorthosites

Fig. 1 ^{40}Ar to ^{39}Ar age spectra for hornblende from the crater bottom (a, H 603) and for suevite ejecta sampled at Otting (b, HO). Each data bar represents one release fraction in the incremental heating procedure, beginning at 450 °C at the left and ending with 1500 °C at the right end. The amount of gas released in the various fractions is reflected in the widths of the bars, expressed as proportion of the total ^{39}Ar contained in the sample. The measured ^{40}Ar to ^{39}Ar ratios are transformed into apparent K–Ar ages using a monitor irradiated together with the samples. H 603 exhibits a nearly perfect age plateau comprising 94% of the total argon release. Sample HO has lost 12.9% of its radiogenic argon from the less retentive sites (lower degassing temperatures), but the plateau age remains undisturbed and is properly defined by the higher temperature fractions, in spite of a shock pressure exceeding 400 kbar.

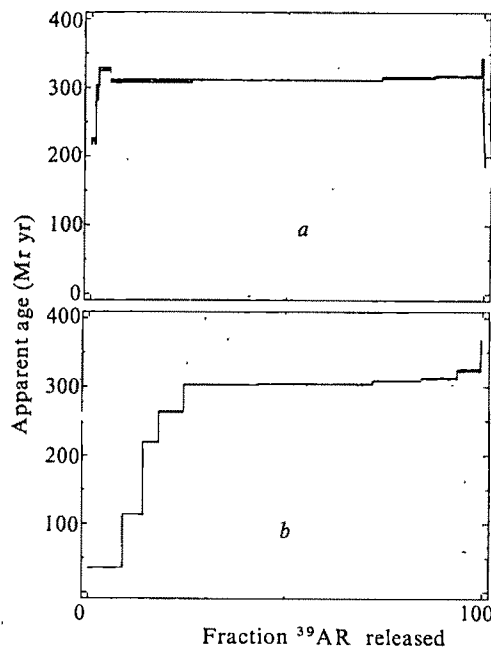


Table 1 Summary of ^{40}Ar - ^{39}Ar results for Ries crater samples

Position*	SAMPLE Parent rock	K [%]	Total $^{40}\text{Ar}_{\text{rad}}$ [$10^{-6}\text{cm}^3\text{g}^{-1}$]	Total $^{40}\text{Ar}_{\text{atm}}$ [%]	Total K-Ar-age [Myr]	Plateau age [Myr]	Plateau range [%- %]	^{40}Ar loss [%]	Shock pressure [kbar]
Near surface samples									
	HMK Amphibolite	0.50	692	3.3	311 ± 11	315.0 ± 10.6	3-98	0.5	300-400
	HO Gneiss, suevite ejecta	0.51	675	11.0	268 ± 5	304.4 ± 4.8	24-94	12.9	400-450
	BO Gneiss, suevite ejecta	4.15	5,200	3.3	290 ± 6	316.6 ± 1.9	24-100	8.9	400-450
Drill core samples above the crater bottom									
	B 377 Suevite breccia	1.89	2,421	4.2	296 ± 3	316.3 ± 2.4	22-100	7.0	n.d.
	H 401 } Amphibolite,	0.92	1,181	3.5	269 ± 3	302.4 ± 3.0	16-75	5.8	< 100
	C 401 } Suevite breccia	3.61	4,514	30.1	290 ± 5	not defined	—	—	< 100
	B 503 } Gneiss, suevite breccia	0.50	625	28.9	287 ± 7	319.3 ± 3.4	28-100	10.8	< 100
	H 570 } Gneiss, suevite breccia	3.95	4,322	2.2	256 ± 5	315.4 ± 2.0	59-100	20.1	200 \pm 50
	H 585 } Gneiss, suevite breccia	0.70	1,059	3.6	316 ± 10	316.6 ± 10.5	3-100	0.2	100-150
	C 1201 } Crystalline rock	0.71	881	5.1	263 ± 6	313.1 ± 5.4	18-98	16.2	< 100
Drill core samples below the crater bottom									
	H 603 Amphibolite, crystalline	0.32	462	3.7	310 ± 5	310.9 ± 4.9	5-99	0.3	< 100
	H 637 Amphibolite, crystalline	0.81	1,134	4.2	309 ± 3	310.5 ± 2.8	2-92	1.1	< 100
	H 1201 } Gneiss	0.67	949	3.5	312 ± 4	314.9 ± 3.7	9-99	1.2	< 10
	C 1201 } Crystalline rock	0.77	1,106	7.7	313 ± 15	316.9 ± 6.2	3-100	—	< 10
Glasses									
	GO Suevite breccia	2.68	166	30.0	14.6 ± 0.3	14.59 ± 0.49	3-92	—	melted
	GM Tektite (moldavite)	2.76	160	22.8	14.8 ± 0.14	14.82 ± 0.15	12-100	1.2	melted

*H = hornblende; B = biotite; C = chlorite; G = glass; MK = Meyer's Keller, crater wall; O = Otting; numbers indicate the depth in metres within the drill core.

Typical sample weights are 200-500 mg, except B 401 (5 mg). The K-content (error $\pm 2.5\%$) follows from the total reactor produced ^{39}Ar and the monitor derived conversion factor. Atmospheric ^{40}Ar is subtracted on the basis of ^{36}Ar (its relative magnitude is given in % of total ^{40}Ar). The total ('conventional') K-Ar age is derived from the integral $^{40}\text{Ar}_{\text{rad}}$ and ^{39}Ar quantities for all temperature fractions. In case of gas loss, it is lower than the plateau age. The error of the plateau age is the standard mean deviation of the ages of the plateau fractions. 'Plateau range' indicates the extension of those fractions which define the plateau age. These entries allow one to visualise the age spectra without explicit figure, examples for HO and H 603 are shown in Fig. 1. The shock pressure is estimated from microscopic inspection following the criteria of Engelhardt *et al.*⁶. Note the absence of a correlation between gas loss and shock pressure. Within the limits of error, essentially all samples yield the same age of $\approx 313 \pm 3$ Myr except the thoroughly molten glasses GO and GM which reproduced the impact age of 14.7 Myr.

from the 30 km Manicouagan structure (conventional K-Ar dating) (ref. 12) and for gneiss and feldspar from the 4 km Brent Crater (conventional K-Ar dating¹³, ^{39}Ar - ^{40}Ar dating¹⁴). This contrasts with the Ries observation of unreddened bedrock ages up to an above 400 kbar (Otting) not only for resistant hornblende but also for biotite. At both the Manicouagan and Brent Craters, samples from a recrystallised melt layer were studied, and probably means that age reductions observed for Manicouagan and Brent Craters are related to a thermal event. From the Ries K-Ar ages we conclude that shock pressure *per se* even as high as 400 kbar does not cause any significant argon loss. Consequently, any eventual age changes caused by meteorite impacts must be related to the temperature elevations associated with the impact and the rate of heat dissipation afterwards.

The peak temperature for the Ries fall-back breccia may be estimated. The common onset temperature, 800 °C, of the age plateaux of crystalline inclusions indicates that the inclusions were never heated above that temperature for any appreciable lengths of time (~ days). This upper limit temperature can be reduced to 600 °C by a model calculation for the cooling history of the suevite layer and an estimate of the hornblende activation energies from the stepwise heating argon release pattern. A 500 °C lower limit has been inferred by Wagner¹⁵ from the thermal stability and the degree of annealing of U-fission tracks in titanite and apatite from the ejected suevite from Otting. Apparently, the Ries ejecta was heated to 550 ± 50 °C at the time of impact.

This work was supported by Gesellschaft für Kernforschung mbH, Karlsruhe.

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Received 7 November; accepted 23 November 1977.

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Alternative ^{210}Pb dating: results from the New Guinea Highlands and Lough Erne

RECENT lake sediments can be dated using ^{210}Pb and fall-out ^{137}Cs . Pennington *et al.*¹, and Robbins and Edgington² have set out the assumptions used in calculating dates and estimating accumulation rates from the declining concentration of unsupported ^{210}Pb in the near-surface sediments of Bleham Tarn and Lake Michigan respectively. In both cases, as in other papers^{3,4}, an essential assumption is a constant initial concentration (c.i.c.) of unsupported ^{210}Pb per unit dry weight in the sediment at each depth, whether or not any variations may have occurred in the rate of accumulation. This assumption requires that in undisturbed cores, unsupported ^{210}Pb concentrations should always decline monotonically with depth. Figure 1 shows unsupported ^{210}Pb concentrations in cores from Lough Erne, Northern Ireland and Lake Ipea, Papua New Guinea. The profiles are 'kinked' and show at one or more points, a marked increase in unsupported ^{210}Pb concentration with depth. The levels at which this occurs in the cores range from 6 to 30 cm. The increases cannot, therefore, be the result of the anomalously low surface concentrations noted elsewhere⁴. Associated biological, chemical and geophysical studies show that the profiles have not been significantly disturbed by physical or biological mixing. These profiles are not consistent with the c.i.c. deposition model and are

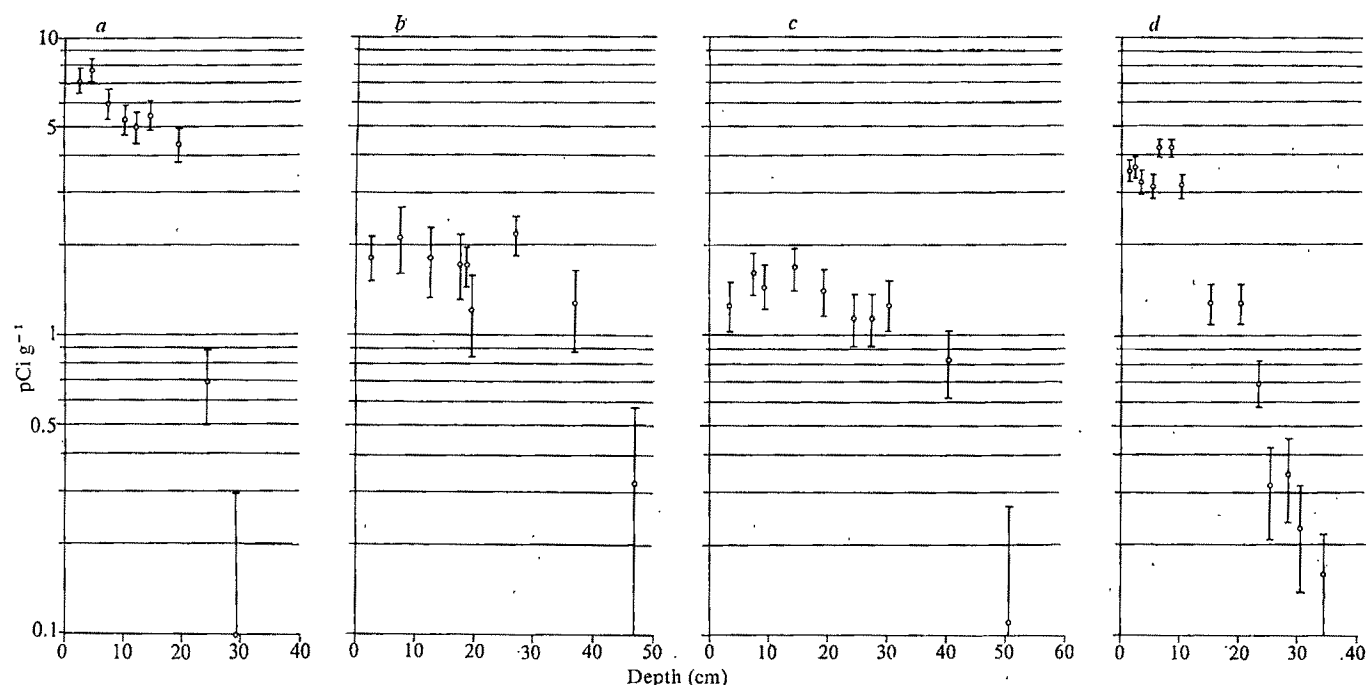
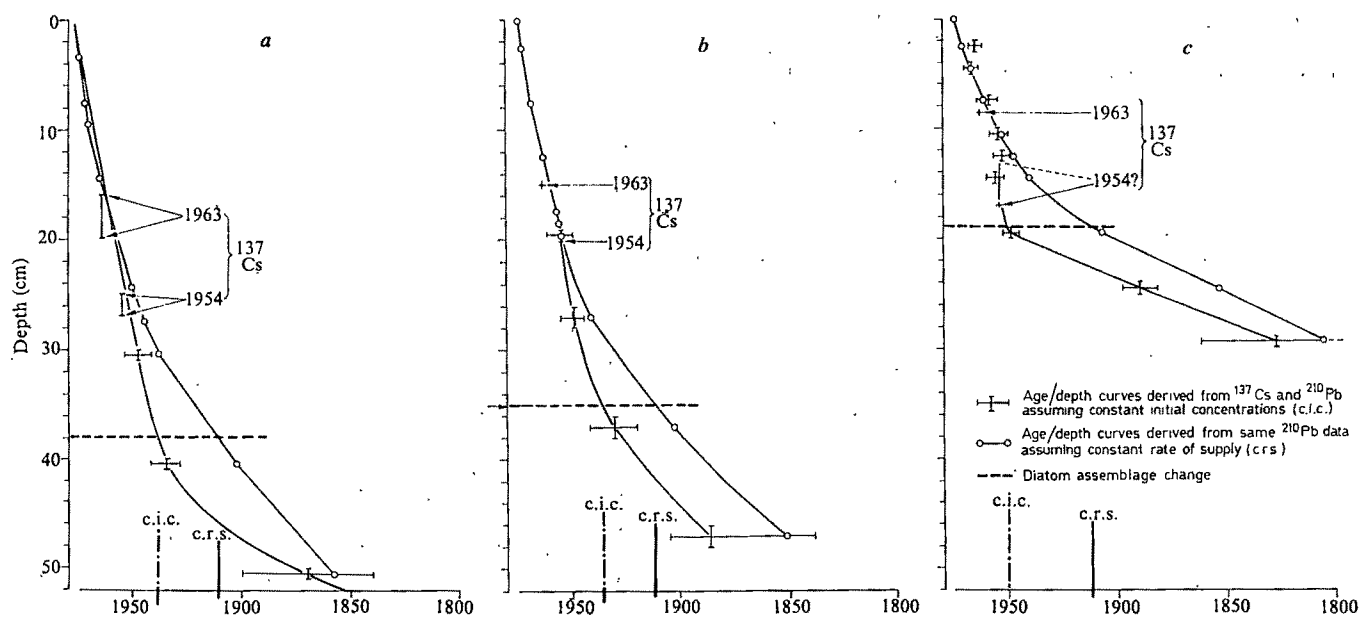


Fig. 1 Unsupported ^{210}Pb concentration against depth in cores from Lough Erne, Northern Ireland, and Lake Ipea, Papua New Guinea. a, Lower Lough Erne, profile SM1; b, Lower Lough Erne profile FM1; c, Upper Lough Erne profile FM2; d, Lake Ipea, Core 1.

regarded as evidence for the dilution of unsupported ^{210}Pb by accelerated sediment accumulation. If such dilution has taken place without leading to a kink in ^{210}Pb concentrations, the assumption of c.i.c. will lead to underestimation of the true age of the sediment below the onset of acceleration. In the case of the 'kinked' profiles, dates are not calculable using the c.i.c. deposition model alone. We have adopted an alternative approach to calculating ^{210}Pb dates using as our main assumption a constant rate of supply (c.r.s.) of unsupported ^{210}Pb to the sediment per unit time and deriving dates from the integrated activity of the radionuclide. Previous authors have referred to the possibility

of calculating dates using this assumption^{5,6} but have not given a full account of the method or evaluated the results of its application. Details of the methods used here are set out elsewhere⁷. This paper compares and briefly evaluates the two alternative models as applied to the sediments of Lough Erne and Lakes Ipea and Egari. The c.r.s. based dates obtained have been compared with those derived either from c.i.c. based ^{210}Pb dates or, in the case of the 'kinked' profiles, from a combination of ^{137}Cs dating⁸ and c.i.c. based calculations. Figure 2 plots the resulting age against depth curves from Lough Erne, Fig. 3, some of the results from Lakes Ipea and Egari.

Fig. 2 Alternative ^{210}Pb based age against depth curves from three cores from Lough Erne, Northern Ireland. The standard errors are shown for the c.i.c. based profiles only. For cores b, FM1 and a, FM2 the strongly 'kinked' unsupported ^{210}Pb concentration profiles above 30 cm (see Fig. 1) make it impossible to derive c.i.c. based age against depth curves above this level. c, SM1. The alternative dates for the diatom assemblage change are marked on the timescale at the base of each plot.



Diagrams of diatom frequency have been completed for all three Lough Erne cores. The main change in diatom assemblages in each core is marked on Fig. 2. In each case, at this stage in the core, the proportion of planktonic diatoms expands and the concentration of frustules (cm^{-3} fresh weight) begins to increase. Using the ^{137}Cs and c.i.c. based age/depth curves, the age of this change ranges from ~1935 in core FM1 to ~1950 in core SM1, both from the Lower Lough. Using the c.r.s. model, dates in all three cores lie between 1907 and 1914.

On the basis of both calculations, sediments at 24.5 cm in SM1 and at 47 cm in FM1 seem to be contemporary. It is therefore possible to normalise these two profiles approximately, with regard to age. The c.i.c. model would require either that the ratio of concentrations at contemporaneous depths in the two sites to be constant or, because the dry weight accumulation rate above this level in FM1 is over three times as fast as in SM1, that the cumulative residual unsupported ^{210}Pb values in FM1 be greatly in excess of those in SM1. The ratio of concentrations between FM1 and SM1 varies from 2.3 : 1 to 4 : 1. Moreover, Fig. 4 shows that

each lake and, in both cases, the first schemes were installed around the turn of the century.

The presence of a synchronous volcanic ash layer in the recent sediments of Lakes Ipea and Egari makes it possible to identify two points at which the sediments for the two lakes are exactly contemporaneous—the surface mud and the mud in contact with the volcanic ash. Assuming a c.i.c. of unsupported ^{210}Pb implies that for all stages of deposition known to be synchronous between the two sites, the ratio between the concentrations of unsupported ^{210}Pb at the two lakes must be the same. Figure 5 shows that this is not the case; for example, in recent times the concentration at Egari has been around three times that at Ipea whereas the ratio is close to one during the period just after the ash fall. Though not compatible with the c.i.c. model this evidence is consistent with a c.r.s. model in which unsupported ^{210}Pb is diluted by more rapidly accelerating accumulation at Ipea than at Egari.

Geochemical studies (Blong, personal communication) have confirmed that the source of the most recent ash fall recorded in the Ipea and Egari sediments, as well as exten-

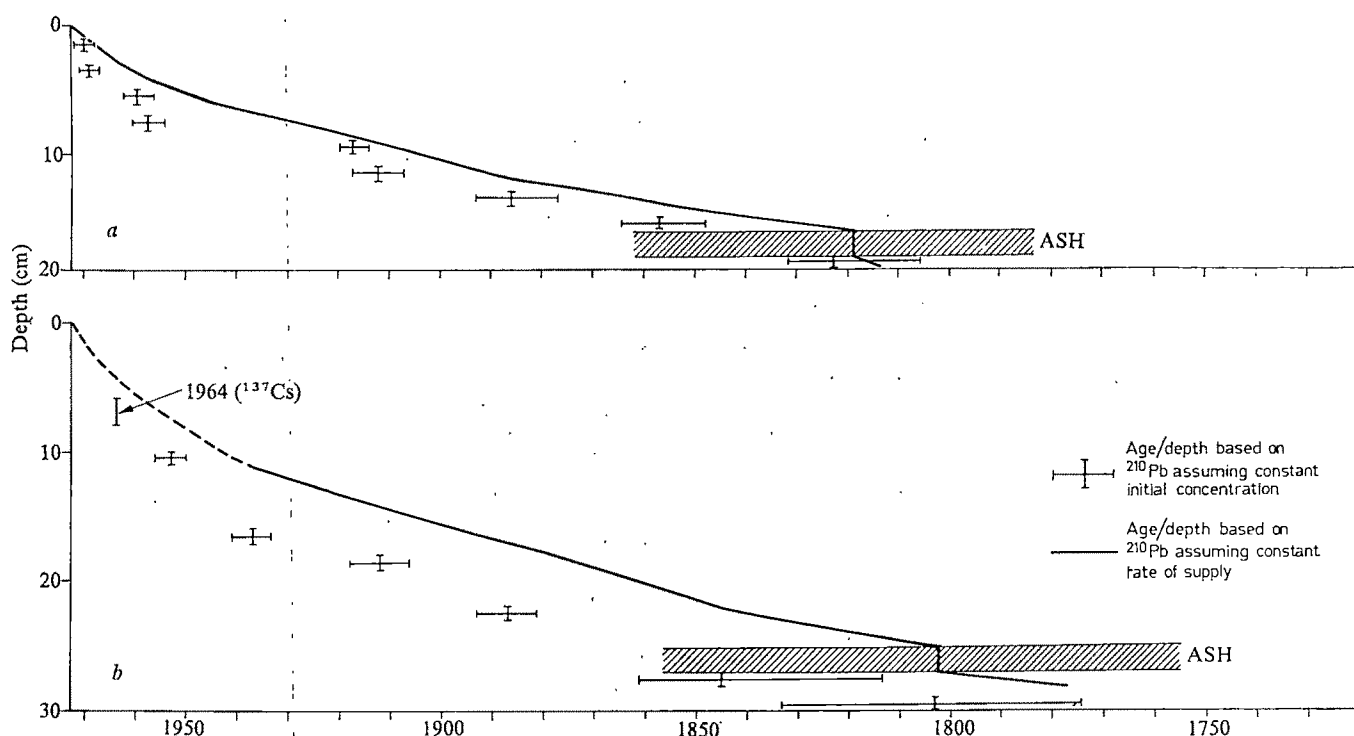


Fig. 3 Alternative ^{210}Pb based age against depth curves for *a*, core 5 from lakes Egari; and *b*, core 4 Ipea in the Highlands of Papua New Guinea. The ^{137}Cs age estimate takes into account the lag in peak fall-out between hemispheres. The upper peaked section of the c.r.s. based age against depth curve from Ipea has been obtained from measurements on core I for which detailed recent data are available; below this level, the data from the parallel adjacent core 4 have been used since at the lowest levels the ^{210}Pb measurements for core I are somewhat high when compared with mutually consistent profiles from the two sites.

the curves and cumulative residual unsupported ^{210}Pb values are almost identical and hence consistent with the c.r.s. model in which the rate of supply of unsupported ^{210}Pb to each site has been comparable, despite the difference in the rate of sediment accumulation.

No firm independent evidence of age is available for any of the recent Lough Erne sediments. The change in the diatom record noted above and redated to around 1910 indicates increasingly eutrophic conditions. It parallels that also dated to the early part of the twentieth century in Lough Neagh⁹. The most likely cause of this is the installation of sewerage schemes within the drainage basins of

sively elsewhere in the New Guinea Highlands, was Long Island some 400 km to the east. Analysis of historical records has shown that the period during which a major eruption in Long Island could have taken place ended long enough before 1827 to have left no obvious major traces on the landscape of the island¹⁰. Since then, the island has been frequently visited and its state well documented by Western observers. Several radiocarbon dates on terrestrial material associated with the ash placed its age at ~250 BP¹¹. Both lines of evidence point to an eighteenth century date and they are quite incompatible with the original c.i.c. based estimates which averaged ~AD1860. The four recalculated

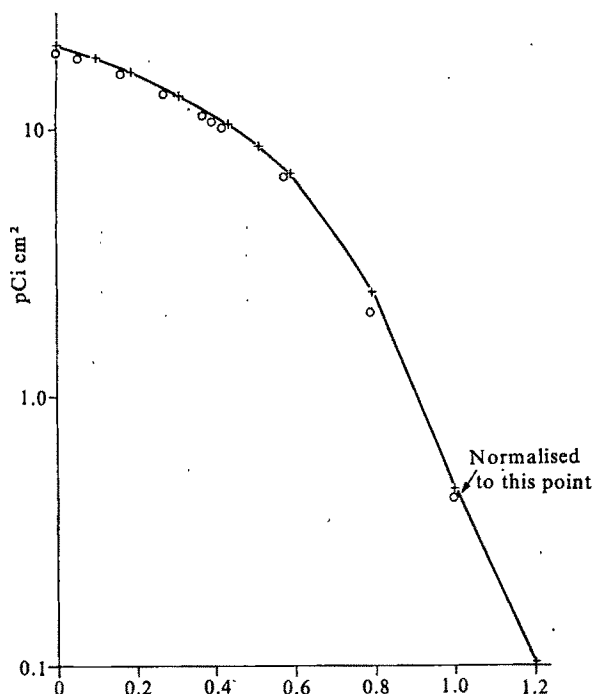
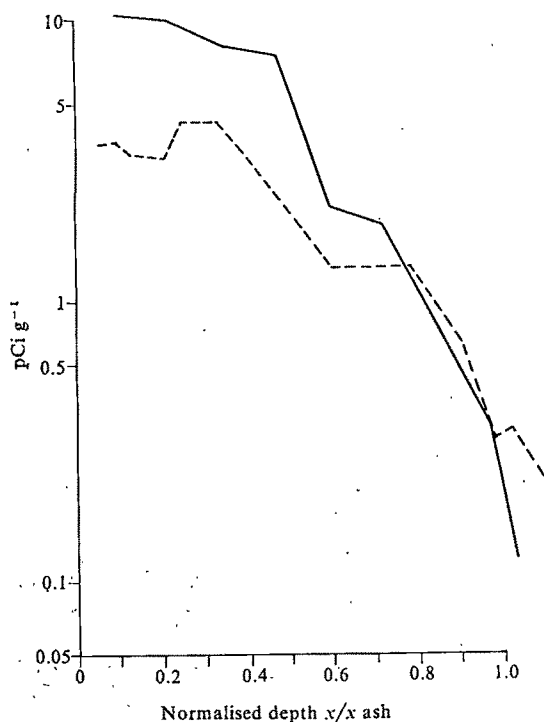


Fig. 4 Cumulative total residual unsupported ^{210}Pb content for Lower Lough Erne cores FM1 (○) and SM1 (+) normalised to a correlated depth.

^{210}Pb profiles from Lakes Ipea and Egari regarded as mutually consistent on internal evidence give an age range between 1800 and 1840 and an average age of 1814. This date is within the time range possible on documentary grounds and much closer to the ^{14}C dates than were the original ^{210}Pb estimates.

Where accumulated rates have not changed over the last 150 years both the c.i.c. and the c.r.s. models will give identical and possibly reliable dates. The data presented

Fig. 5 Unsupported ^{210}Pb concentrations from Egari core 5 (solid line) and Ipea core 1 (dashed line) normalised for depth (×) by the synchronous volcanic ash layer common to both sediment profiles.



here suggest that in a lake where accumulation rates have accelerated, the c.r.s. model may provide dates which are more consistent on internal grounds and more compatible with existing external evidence.

We thank NERC, the Nuffield Foundation and the Department of the Environment, N. Ireland for financial assistance. We also thank J. D. Eakins and R. S. Cambray for the ^{210}Pb and ^{137}Cs profiles and R. Blong for encouragement.

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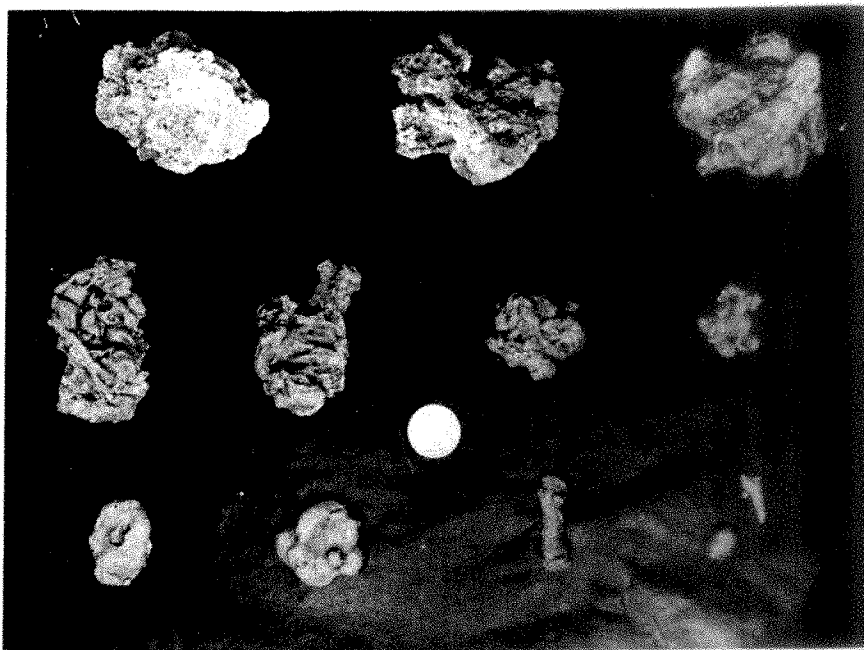
Subaqueous sulphur lake in Volcan Poas

GREENISH amorphous sulphur particles are present at all levels immediately around the crater of Volcan Poas. The eruptive plumes of the mild submarine volcanism which characterises this volcano, are charged with these particles. We suggest here that they are strong evidence that a lake of molten sulphur exists, or is formed temporarily before an eruption, below the existing lake of water in the crater. We have collected many samples of greenish amorphous sulphur, with various quantities of admixed ash from regions all around the crater, noting some concentrations in the east-west prevailing wind directions. Figure 1 shows sulphur specimens and their morphology; Fig. 2 gives a profile of the crater; Fig. 3 shows approximate outlines of areas in which sulphur samples are found. Table 1 gives approximate analyses of representative samples.

Volcan Poas has been in a state of mild eruptive activity for about two centuries. Its usual activity is similar to shallow submarine volcanism. At intervals ranging from several times a month to several times an hour a plume arises from the approximate centre of the crater lake and ascends to heights varying from a few metres to 500 m or more. The plume is laden with particulate matter and may appear black or greenish. At much longer intervals, more violent eruptions producing grey ash, scoria, plastic lavas and bombs will occur. The most recent of these occurred in November 1976, and spread a mantle of grey ash for some kilometres to the west of the crater.

We shall look at the plume eruptions and their similarity to submarine volcanism, and we shall explain them using an approach¹ which may be generally applicable. All active volcanic structures contain a stack, or pile, of immiscible liquids separated by more or less definite boundaries; within each level processes of heat and mass transfer may occur by conduction and convection; bubbles may form, grow,

Fig. 1 Samples of amorphous sulphur from Volcan Poas. Ash content high in sample at upper left. Coin diameter = 1.5 cm.



rise and attach to upper boundaries, or disappear; processes of dispersion, sedimentation and crystallisation develop within layers. Across boundaries, or in boundary layers, fluxes of mass, heat, bubbles, crystals, and so on may occur. When the local vapour pressure within a particular level exceeds the hydrostatic pressure at that level, and the temperature distribution above will permit the growth of a vapour-filled cavity, then a suitable disturbance may initiate the vaporisation expansion wave which supports the growth of a plume, and an explosive eruption will take place.

In the present example, we consider the volcanic pile to be similar to that for shallow submarine volcanism. This assumption implies a volcanic pile with dry magmas below, more volatile magmas above these, then a crust or plastic layer, water-rich muds and clays, and water suspensions grading from heavily laden to lightly laden in the upward direction.

In Fig. 1 the sulphur samples all show evidence of a fluid dynamic eruptive process. The plume gases entrain and interact with molten or plastic, liquid sulphur by means of the reverse flows around the deformable fluid particles being accelerated by the vertical jet in the plume. Representative particles of the collection show elongations, separations into branches with ball-like terminations, twisted streamline configurations, deformed spheres, tiny spherical droplets, plastic masses with considerable ash content; and there exist some of all these types with vesiculated surfaces and interiors. These latter were probably burning during the eruption, over at least part of their trajectories.

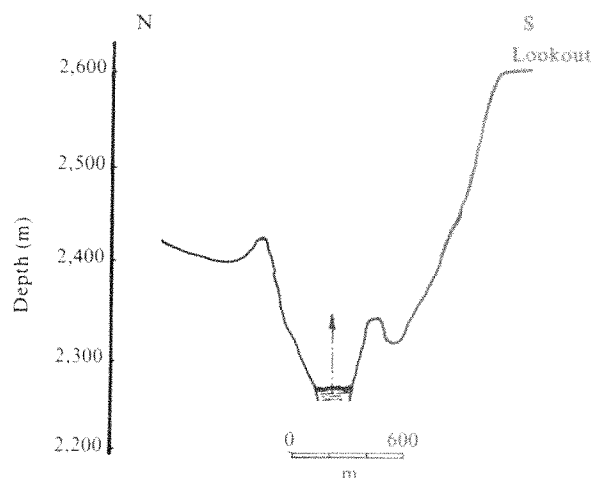
Experiments in which existing pieces of our collection are set on fire show a clear blue flame characteristic of sulphur, produce SO_2 fumes, and leave very little residual ash. We observe vesiculation both of the surface and of the interior under the flame.

According to accounts of the rangers at Volcan Poas National Park, bushes at the high plateau level, 300–500 m, above the lake, were set on fire. We take the region devoid of living vegetation as the area covered, during various eruptions, by sulphur particles. In a circle of radius 1/2 mile around the lake centre, the density of sulphur particles suggests that several hundred tons of sulphur are lying on the surface or have been washed into the river courses by the heavy rains of the wet season.

The distribution with height, and the change from high sulphur content at lower altitudes, to less sulphur and more ash at the upper altitudes above the lake suggests that the distribution in the columnar plume reflects a similar distribution in sulphur content of the portion of the volcanic pile where sulphur occurs.

We believe that the evidence available justifies the assumption that there is a level, or lake, of molten sulphur below the existing lake of water. The specific gravity of sulphur, 1.9–2.0, is intermediate between that of andesitic magma, 2.5–2.7, and that of pure water. We have no evidence for the average specific gravity of a water-rich clay or mud of volcanic ash, but we assume that values intermediate in the range 1.0–2.0 can be encountered. Therefore, to our model of the volcanic pile we add, immediately above the crusted magma, a layer of molten sulphur on which floats a layer of water-rich volcanic ash and lapilli. Above this layer lies the crater lake which from its greenish appearance carries a suspended load of sulphur and ash varying with time. One of the precursor signs of an imminent eruption is the appearance of a grayish spot, 25–50 m in diameter, in the centre of the lake. We take this to be

Fig. 2 Profile of the crater. Arrow indicates the position of the plume.



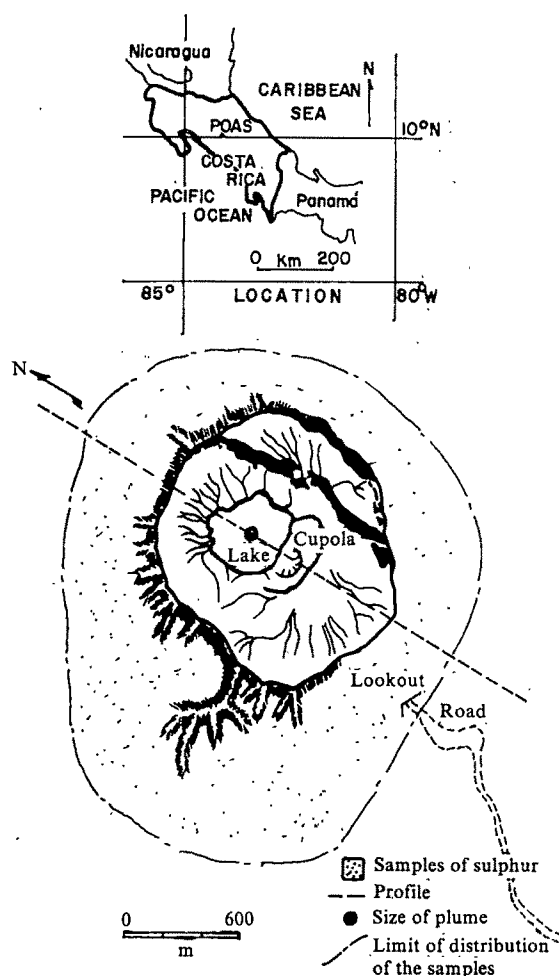


Fig. 3 Map of the crater.

an indication of convective processes that carry portions of the underlying sulphur-ash levels to the surface.

We can only speculate how the plume forms and the eruption occurs. Because the plume is laden with molten sulphur-ash particles some of which catch fire on exposure to air, we may presume high temperatures in the sulphur layer, probably higher than the normal boiling point, 444 °C, in the lowermost portions. If the propulsive fluid for the eruption is sulphur vapour, then temperatures much higher than the boiling point will be encountered at the level between solid or plastic magma and molten sulphur.

A possible mechanism for production of an eruption, other than the gradual increase in temperature of the column during the hydrostatic phase, would be an overturning instability brought about by an increase in density of the mud deposited by sedimentation from above. As the density of the overlying mud increases to near equality with that of the molten sulphur, an overturning instability would be possible that would rapidly expose high temperature sulphur to overlying water and bring about an explosion similar to those noticed in fuel-coolant interactions².

Table 1 Analysis of sulphur content of representative samples

Sample	1	2	3	4	5
Weight % sulphur	16	85	98	98+	99

We think that other sulphur producing volcanoes, particularly those of andesitic type, should be examined for signs of the existence of a lake of molten sulphur in the volcanic pile.

S.M.R. is a visiting professor in the cooperative programme between the governments of Italy and Costa Rica.

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The Soufrière Crater Lake as a calorimeter

IN 1971-72, $8 \times 10^7 \text{ m}^3$ of basaltic andesite lava were extruded into the summit crater of Soufrière volcano, St Vincent¹. The first half of the eruption took place entirely subaqueously and the second half almost entirely so and these facts have provided us with an unusual opportunity to carry out quantitative studies of several aspects of the eruption. Before the eruption the crater contained $7.5 \times 10^7 \text{ m}^3$ of water and the potential of this lake as a giant condenser of volcanic gases has already been demonstrated². We show here that by treating the crater lake as a giant calorimeter we can make reasonably accurate estimates of the rate at which heat was lost by the erupted lava and hence draw important conclusions about its mode of extrusion. The heat transfer data are consistent with the idea that the lava mass accumulated by the multiple extrusion of numerous small lava bodies¹ rather than by extrusion of a single domelike mass, as has been proposed by other workers^{3, 4}.

The eruption of 1971-72 began some time in early October 1971 and until November 20, 1971 it continued entirely subaqueously. Before the eruption the crater lake contained $7.5 \times 10^{10} \text{ kg}$ of water at a temperature close to the mean ambient temperature of 22 °C. Seepage into the crater walls and normal evaporation were balanced almost exactly by precipitation. By the time that lava began to emerge through the surface of the lake on 20 November 1971, the mass of water in the lake had decreased to $5.7 \times 10^{10} \text{ kg}$. The temperature at the edge of the lake was 82 °C on 2 November and probably was maintained at at least this value for at least 1 month before 20 November. Throughout this early part of the eruption the temperature of the streams and springs on the outer flanks of the volcano was monitored both by aerial infrared photography and by manual measurements and we found no evidence that seepage from the lake had increased. We therefore conclude that $1.8 \times 10^{10} \text{ kg}$ of water were lost by excess evaporation caused by heat input from the lava. Heat supplied to the crater lake up to 20 November therefore includes four main components. (1) The heat required to raise $7.5 \times 10^{10} \text{ kg}$ of water from 22 °C to 82 °C ($1.9 \times 10^{16} \text{ J}$). (2) The heat required to raise $1.8 \times 10^{10} \text{ kg}$ of water to the boiling point at the elevation of the lake surface (98 °C) ($0.1 \times 10^{16} \text{ J}$). (3) The heat required to convert $1.8 \times 10^{10} \text{ kg}$ of water to steam at 98 °C ($4.1 \times 10^{16} \text{ J}$); and (4), the heat lost from the surface of the lake to the atmosphere by radiation and convection. The radiative heat-loss can be estimated from the Stefan-Boltzman equation

$$\delta H / \delta t = \sigma \Sigma (T_s^4 - T_o^4) W \text{ m}^{-2}$$

where σ is Stefan's constant, Σ is the emissivity of the water surface and T_s and T_o are the surface and ambient absolute temperatures respectively. Inserting appropriate values for the variables and assuming that the lake reached 82 °C 1 month ($2.5 \times 10^6 \text{ s}$) before 20 November the radiative heat loss is $0.1 \times 10^{16} \text{ J}$. Similarly the heat lost by convection can be estimated from the approximate expression (ref. 4):

$$\delta H / \delta t = 1.52 (T_s - T_o)^{1/3} W \text{ m}^{-2} (\text{°C})^{-1}$$

which gives a convective heat loss of $0.1 \times 10^{16} \text{ J}$. The total

heat supplied to the lake water between the beginning of the eruption and 20 November was therefore at least 6.3×10^{16} J. This value is certainly an underestimate since it excludes heat lost by conduction into the crater walls and by convective transfer to pre-existing groundwater in the crater walls.

The mass of lava erupted up to 20 November was 10^{11} kg. The available thermal energy of the lava includes the enthalpy of melting (latent heat) and the heat lost by the lava in cooling from its eruptive to its final temperature. Since the lava pile was still growing on 20 November it is clear that it retained a fluid core which was probably still at the eruptive temperature of approximately $1,050^\circ\text{C}$ whereas the outer surface of the lava could not have been at a temperature greater than 100°C . There was therefore a carapace of solidified, though perhaps extensively fractured, lava separating the fluid core from the lake water with a temperature difference of 950°C across the carapace. Any physically reasonable heat-transfer mechanism, either by conduction or by circulation of fluids, requires that the temperature gradient across the carapace should be at least quasilinear which implies that the mean temperature of the carapace was the arithmetic mean of the water and lava temperatures. If the mass of lava forming the carapace was M kg, the total amount of heat lost by the lava in solidifying and cooling from $1,050^\circ\text{C}$ to 575°C was

$$H = M[L + 475C]$$

where L is the latent heat of the lava and C is its specific heat capacity. Taking $L = 3 \times 10^5 \text{ J kg}^{-1}$ and $C = 10^3 \text{ J kg}^{-1} (^\circ\text{C})^{-1}$, then

$$H = 7.75 \times 10^5 M \text{ J}$$

Equating the heat lost by the lava to the heat gained by the lake we have

$$M = 8.1 \times 10^{10} \text{ kg}$$

That is, of the lava erupted up to 20 November over 80% had solidified and cooled to a mean temperature 475°C below its eruptive temperature by that date. We emphasise that this is a lower limit, because the heat supplied to the lake water is known to be an underestimate. A previous assessment of the heat budget² did not take into account all the factors considered here and was an even greater underestimate of the volume of cooled lava.

The same pattern continued during the rest of the eruption. When extrusion of lava stopped on 20 March 1972 a total of 2×10^{11} kg of lava had been erupted, and 5×10^{10} kg of water had evaporated. By calculations similar to those above we estimate that about 90% of the lava had solidified when the eruption ceased. This again is a lower limit since we were unable to make accurate estimates of the heat lost by radiation directly from the lava to the atmosphere after it emerged from the lake. These observations therefore make it clear that during the 1971–72 eruption of the Soufriere of St Vincent the lava solidified at about the same rate that it was erupted and that the solidified lava cooled considerably after crystallisation.

During the eruption, large quantities of heat were transferred rapidly from the lava to the lake water. The characteristic dimension of a typical lava cooling unit can be estimated from the observation that the lava was able to lose a significant proportion of its heat content to its surroundings within a time period which was less than 1 month. The thermal time constant τ of a body with characteristic dimension R metres and thermal diffusivity $\kappa \text{ m}^2 \text{ s}^{-1}$ is of order R^2/κ . Putting $\tau = 1$ month ($2.5 \times 10^6 \text{ s}$), $\kappa = 10^{-6} \text{ m}^2 \text{ s}^{-1}$ we estimate that the maximum possible characteristic dimension of the individual cooling units was in the range 1–5 m. This result clearly excludes the possibility that the lava mass was extruded as a viscous dome^{3,4} with a characteristic dimension of order 500 m. There seem to be two possible ways in which the intimate mixing of lava and water necessary to cause the observed rapid heat-flow could have been achieved. The first involves breaking the whole

lava pile into individual blocks with diameters less than 5 m. This possibility can be excluded simply on the grounds of visual observations at the time of eruption¹. There were indeed rock avalanches during the later stages of the eruption but these involve only a very small proportion of the erupted mass. The second possibility, which is supported by the visual observations, is that the lava pile grew by the successive extrusion of overlapping flow-units, each with a characteristic dimension (which in the case of flow is its thickness) of order 1–5 m.

The combined field observations¹ and calorimetric evidence show that the 1971–72 eruption was a quiet extrusion of a lava flow. The gross morphologic form of the lava mass was controlled by the shape of the container (crater) and the cooling effect of the surrounding lake water, and not by the viscosity of the lava. Other features of the eruption, particularly the lack of associated seismicity, the absence of explosions and the composition of the lava suggest that the viscosity of the magma was low and the results presented here show that the gross morphology of the lava pile is consistent with this conclusion.

This eruption marks the first documented extrusion of a lava flow in the Lesser Antilles island arc. Previous events of extrusion of minor lava masses within the Soufriere crater lake are, however, indicated by very sketchy historic accounts¹, suggesting episodes of minor lava extrusion at intervals between the major explosive eruptions of this volcano. The fact that the Soufriere is capable of quiet lava flow eruptions as well as violent explosive ones must be considered in any theory of its eruptive mechanism.

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Circular structures of large scale and great age on the Earth's surface

THE Earth's surface exhibits faint circular patterns which have not been described before. These circles are characterised by near perfection of outline, by the presence of topographic highs (rims) along parts of their circumferences, by their generally large scale (diameters of from under 7 km up to approximately 700 km in the areas examined), and by their definition in various geological environments, in many rock types, and in rocks of all ages. Many of the circles are intermittent in places along their rims but about 55% of the approximately 1,170 definite circles observed to date can be visually traced around an entire 360° of arc. The circles are further characterised by the presence of fracturing and brecciation along parts of their rims and by the extraordinary control they place on regional geology in general and on ore mineralisation in particular.

To date, circles of this nature have been observed clearly in several areas with ancient continental crust at depth: the western United States, northernmost Mexico, the Appalachians, Alaska and the Yukon. Their existence is also strongly indicated in Madagascar and Corsica. No other areas have been examined. The circles are visible on displays which were produced from commercially available raised plastic relief maps

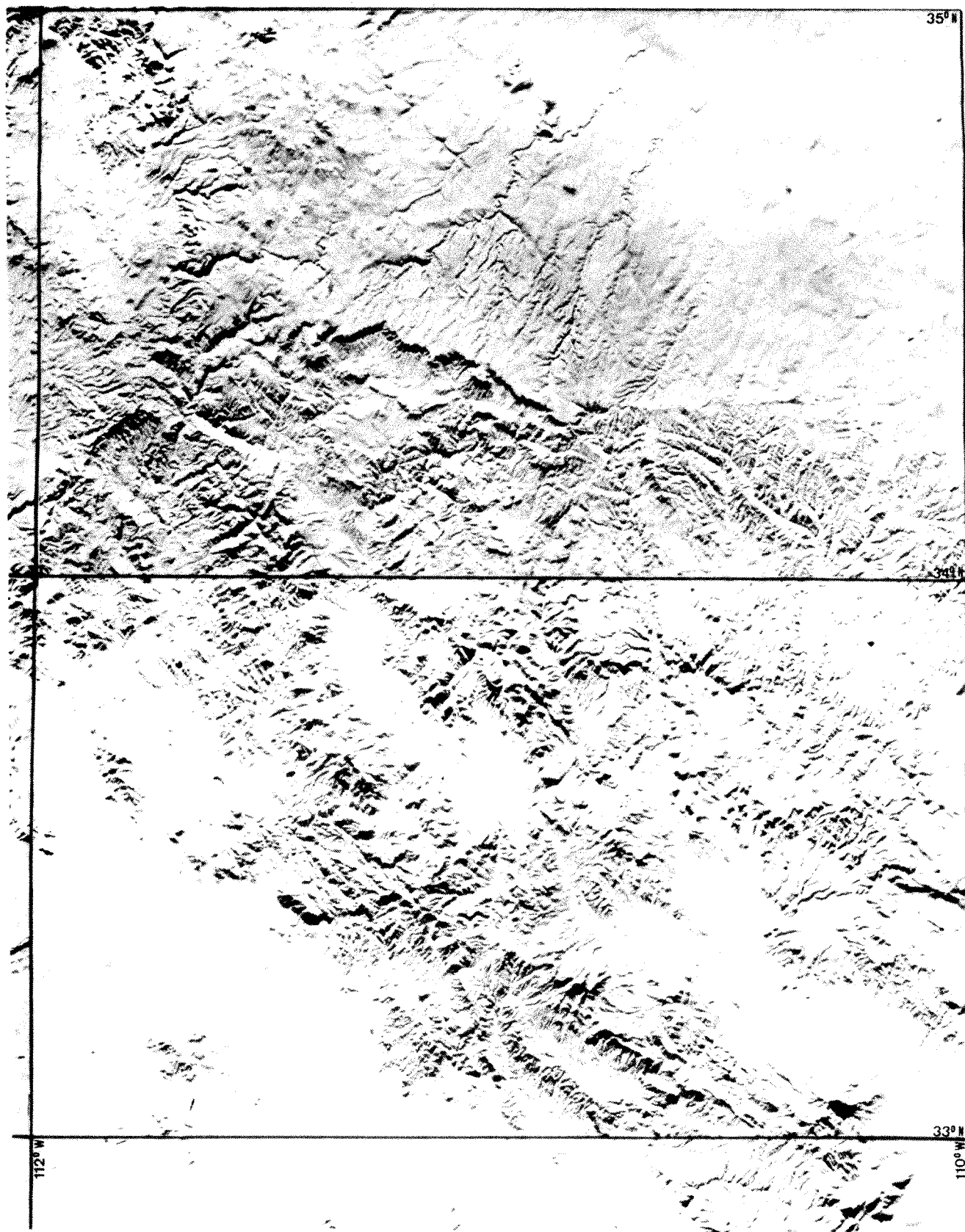


Fig. 1 Portion of Arizona at a scale of 1 : 1,000,000 produced by the method outlined in the text. The original relief maps were sprayed white and had twofold vertical exaggeration. The test area is bounded by thick latitude and longitude lines and the extra areas to the west and south are included to facilitate viewing of some of the circles.

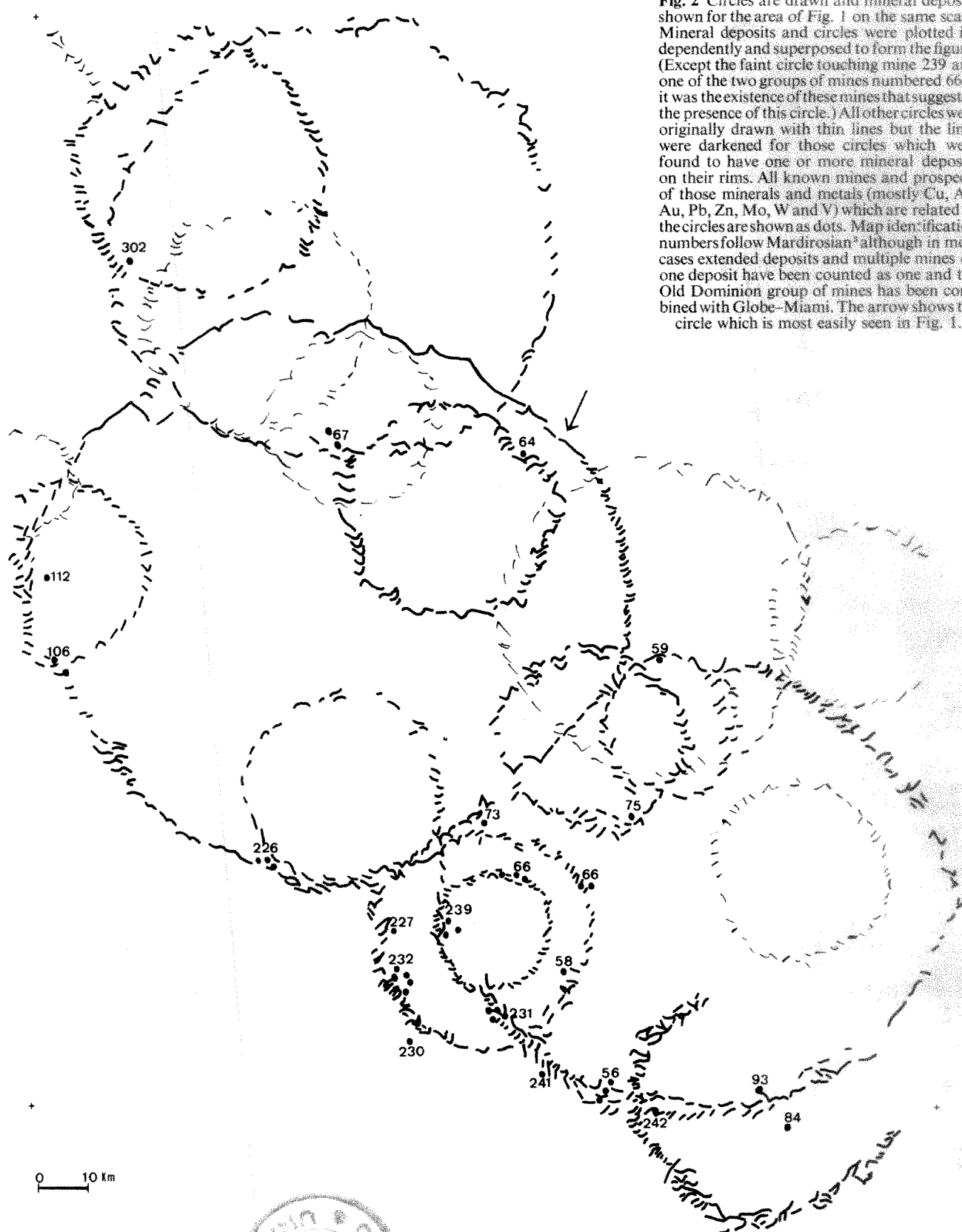


Fig. 2 Circles are drawn and mineral deposits shown for the area of Fig. 1 on the same scale. Mineral deposits and circles were plotted independently and superposed to form the figure. (Except the faint circle touching mine 239 and one of the two groups of mines numbered 66—it was the existence of these mines that suggested the presence of this circle.) All other circles were originally drawn with thin lines but the lines were darkened for those circles which were found to have one or more mineral deposits on their rims. All known mines and prospects of those minerals and metals (mostly Cu, Ag, Au, Pb, Zn, Mo, W and V) which are related to the circles are shown as dots. Map identification numbers follow Mardirosian² although in most cases extended deposits and multiple mines on one deposit have been counted as one and the Old Dominion group of mines has been combined with Globe-Miami. The arrow shows the circle which is most easily seen in Fig. 1.

Table 1 Lode occurrences in the Arizona test area of those metals and minerals whose emplacement is controlled by the circles

District, mine, area or deposit	Principal commodities
Banner (Xmas, Troy, Dripping Springs, Hayden) (56)*	Cu, Pb, Zn, Ag, Au, Mo
Bobtail, Samsel (58)	W
Canyon Creek (59)	Turquoise
Ellison (64)	Cu, Pb, Zn, Ag
Globe (Globe-Miami); and the Old Dominion group of mines† (66)	Cu, Ag, Au, Pb, Zn, Mn, Fe, V, turquoise, chrysocolla
Green Valley (Payson), 2 deposits (67)	Au, Pb, Zn
Pinto Creek (Pinto Valley) (73)	Pb, Cu
Richmond Basin (MacMorris, McMillen) (75)	Ag
Aravaipa (84)	Pb, Zn, Cu, Ag, Au, Mo
Stanley (Stanley Butte, Quartzite Mountain) (93)	Cu, Pb, andradite garnet
Cave Creek (Gold Cliff, Golden Reef), 2 deposits (106)	Au, Cu, Ag, Pb, Mo, V, W
Magazine, Greys Gulch Mine (112)	Cu, Ag, Au
Goldfields (226)	Au, Ag
Hewitt Canyon (227)	Serpentinised limestone
Martinez Canyon (230)	Pb, Ag
Mineral Creek (Ray, Kelvin) (231)	Cu, Au, Pb, Zn, Mo
Mineral Hill (232)	Au, Pb, Zn
Pioneer (Magma, Silver King, Superior) (239)	Cu, Ag, Au, Pb, Zn, Mn
Riverside (Kearney, Rare Metals) (241)	Au, Ag, Cu, Pb, Mo
Saddle Mountain (242)	Pb, Ag
Squaw Peak (302)	Cu, Mo

The data shown are taken from Mardirosian³.

*The figures in parentheses are reference numbers taken from ref. 3 and are also used in Fig. 2.

†The Old Dominion mines have been added and are grouped with Globe-Miami.

with a 1 : 250,000 scale and a two- or threefold vertical exaggeration. These were illuminated at a low angle by a parallel light source and photographed individually. Photographs of adjacent maps were joined to give regional coverage^{1,2}. Figure 1 is an example of such a display and covers a part of Arizona.

The area shown in Fig. 1, extending from 33° to 35°N and from 110° to 112°W, covers several mining districts and was selected as a test area in which to examine the relationship between circles and mineralisation. Known mineralisation in this area includes 24 mappable occurrences or deposits of copper, silver, gold (other than alluvial), molybdenum, vanadium, tungsten, zinc, lead (other than lead occurring with alluvial gold), iron (where associated with other metals), manganese (where associated with other metals), serpentinised limestone, and turquoise. Most of the deposits are polymetallic and four are major porphyry deposits (Globe-Miami-Old Dominion, Magma-Silver King-Superior, Ray-Kelvin, Christmas-Troy-Dripping Springs-Hayden). Table 1 gives additional details. Nineteen circles were located within or partly within this test area and forty measurements of the apparent rim widths of these circles gave an average value of 2.1 km.

Using this rim width, it was found that twelve of the nineteen circles had at least one known mineral deposit along their rims and that all 24 mineral occurrences or deposits of the types listed above fell 'on' or 'very close' to a circular rim. These two terms correspond to the two highest ratings in the five point semi-quantitative scoring system used. Most of the 24 deposits were in the 9% of the total area which was classified as 'on' a rim (see Fig. 2).

The circles are not easy to see, even on the original photographs, and it is possible that a few of the faintest circles have no objective reality. There is, however, a positive correlation between degree of visibility and mineralisation. The five most visible circles have 16 out of the 24 mines or deposits associated with them. The five next most visible circles have 11 mines or deposits on or near them, 7 of which are also on or near one of the most visible circles. The nine least visible circles have only 3

deposits on them, all of which are also on or near a more readily visible circle. The degrees of visibility were assigned by J. B. Southard of the Massachusetts Institute of Technology without reference to the presence of mineralisation. One additional faint circle with mineralisation was found after the assignment of the degrees of visibility, and numerous other faint or doubtful circles were noticed subsequently. The portion of the test area covered by rims is increased from 9% to 42% if all of these are accepted.

Occurrences in the Arizona test area of asbestos, barite and iron (when not associated with other metals) also seem to bear some relationship to the circles. There was no correlation between the circular rims and the locations of deposits of uranium, manganese (in monometallic ores), perlite, placer gold, mercury, gypsum, sand and gravel, sandstone, limestone, peridot, quartz crystal, amethyst, jasper and onyx marble. No other occurrences of economic minerals or stones are reported from the area with the exception of fossil fuels³. A brief survey of circular rims found in the Butte, Montana area also showed a strong correlation with mineralisation, so the striking results from Arizona are not unique.

The correlation of mineralisation with the circles stands on its own and does not depend on the acceptance of any particular theory as to how the circles came into existence.

Individual circles exhibit different degrees of visibility. In general, a circle that is readily visible along one portion of its circumference will be readily visible over the whole circumference, and a faint circle will be uniformly faint. The great majority of circles are indeed indistinct, and there is a real problem in reproducing the same circles in different viewings of the photomaps. Larger circles are easier to see than the smaller circles and a positive correlation between circle size and presence of mineralisation is suggested. This may have an upper limit, however. In this study no major mines were noted on circles larger than 150 km in diameter. Much larger circles may also exist. A circular feature of generally similar appearance and a 2,200-km diameter seems to encircle the southern end of Africa, passing through the watershed area of central Angola, the Limpopo Valley region, the offshore edges of the Mozambique and Agulhas Plateaus, various seamounts and the Walvis Ridge.

Intersections of two circles seem to have a somewhat enhanced chance of being mineralised and thus the identification of the fainter circles may prove important after all. In a few cases, the most notable of which is the Bingham copper mine in Utah, mineralisation was found at the intersection of a circle and an obvious linear feature.

The centres of the circles seem to fall in a northwesterly trending pattern in Arizona and in a northeasterly trending pattern in the Appalachians, in both cases forming elongated clusters parallel or nearly parallel to the regional geological trends. Circles are much more abundant in mountainous areas than in the adjacent plains in many regions.

The positions of many towns, roads, railroads, rivers, lakes and reservoirs seem to be related to circles and this is probably a natural consequence of the fact that the circles are defined by topographic criteria.

There is little or no correlation between circles seen on the displays used in this study with circles seen on ERTS photography.

A final observation offers an essentially independent verification of the features discussed here: when transparent overlays with circles plotted on them are reproduced at the correct scales and placed over geological maps of the corresponding areas, the various colours representing different rock types fall into new patterns which, although previously unperceived, either on the maps or in the field, are real and require interpretation. Figure 2 can be enlarged to make a suitable overlay for the Geological Map of Arizona⁴.

The circles can be interpreted as having been formed by the impacts of meteorites during the most recent major bombardment of the Earth. At the time of this bombardment, which

by analogy with the moon, was a discrete episode approximately 4,000 Myr ago, the Earth already had a brittle crust. Estimates of the thickness of that crust vary greatly. The Earth today is brittle down to a depth of at least 2.5–4 km; below this it is ductile. Impacts can cause major fracturing through to the brittle/ductile transition depth and the resulting scars may never heal completely due to a combination of several mechanisms. For instance, as the impact site is eroded away, the ductile subbasement will rise and become brittle with decreasing pressure, and the circular scars will be propagated downwards to the new brittle–ductile boundary. Similarly, movements in the fractured zones can be generated upwards to bequeath the circular patterns to overlying sediments or igneous sequences^{5,6}. A history of this nature seems capable of accounting for the preservation of some of the circular scars throughout geological time. The present-day fracture patterns may thus be inheritors or descendants of the original impact craters rather than direct remnants of the craters themselves. The circles differ from previously described astroblemes and the usual criteria of shock metamorphism have not been used for their recognition.

The process of sequential inheritance, by which the geological structures remain after the rocks in which they were formed are gone, is proposed as the mechanism which permits 4,000-Myr-old circular structures to exist in profusion on a planetary surface which holds virtually no rocks thought to be of such antiquity. The geological structures remain after the rocks in which they are formed are gone—an analogy to the smile of the 'Cheshire Cat' comes to mind. Alternative hypotheses avoid this problem but give rise to others.

(1) If the circles are interpreted as artefacts of the map maker, the correlation with mineralisation is unexplainable.

(2) Although some of the circles may be modified calderas (there is evidence suggesting that some Tertiary and Quaternary calderas may have inherited and taken over pre-existing circular sites), the circular scars have a systematically higher degree of circularity than is typical of recognised calderas, and caldera complexes and their metamorphosed equivalents would have to be very much more widespread than present geological maps indicate.

(3) If the circles have been generated from below at various times as, say, super ring-dikes, or their extrusive counterparts, why are their rocks not generally recognised as such and mapped appropriately? In addition, the apparent absence of super ring-dikes in fresher states of preservation would seem to go against the principle of uniformity. The super ring-dike explanation is also difficult to reconcile with the observation that there are few circles smaller than 7 km in diameter and none under 5 km. On the other hand, the small scars caused by impacts which did not break through to the brittle–ductile boundary would have healed over completely in time. Partially healed astroblemes might exhibit features which are, nevertheless, similar in appearance to those of large ring dikes.

Difficult questions remain even if the impact scar origin for the circles is accepted. For instance, the trends which parallel the regional geology in Arizona and the Appalachians have no clear interpretation, nor does the observation that the circles are found in greater numbers in the mountains than in the surrounding plains. Two useful speculations concerning these observations are: first, that the presence of the circular structures permitted or even caused the mountains to form where they did, or, alternatively, that the forces that formed the mountains also reopened the old scars and, second, that the limited sensitivity of the map making or photographic techniques used hinders the identification of circles in regions of low relief.

Geological activity after the formation of the circular features might have prevented their complete healing over and thus: (1) permitted the intrusions of the igneous bodies which are partly responsible for the topography of the rims, (2) permitted the introduction of mineralising fluids from below, (3) permitted the introduction of descending and oxidising surface waters from above, and (4) maintained the circular patterns on the Earth's surface.

Two main characteristics of 'porphyry' copper deposits are association with an intrusive body and, in almost all cases, supergene enrichment of the ore zone. A third characteristic is dependence on intense fracturing whose nature has not been satisfactorily explained previously^{7,8} despite the observation by Godwin in 1973 that "some breccias and adjacent rocks in the vicinity of porphyry deposits . . . have features that seem indicative of shock metamorphism"⁹. This study has shown that a fourth characteristic of porphyry deposits in the Arizona test area and elsewhere is that they are confined to circular rims. All these characteristics can be explained as consequences of an impact-scar site of formation.

If the circles are formed by impacts, they will be surface expressions of forms which, due to geometrical constraints, are generally cylindrical or perhaps somewhat conical. These are most probably essential elements of the "deeply penetrating fundamental 'plumbing' systems" which Jerome and Cook¹⁰ and others believe exert a controlling influence on the concentration of metals. For the case of the porphyry type deposits, it is evident that the circular patterns and their extensions in depth did influence the movements of various fluids, hot, cold, rising, descending, viscous, non-viscous, recent and ancient, and this suggests why other types of deposits which are formed partly through the action of thermal waters (gold-bearing quartz veins, for instance) should be preferentially located on the circular rims. A logical extension of this concept and some fragmentary evidence indicate that the scar structures also affect the distribution of other fluids, groundwater, gas and oil.

I thank Renée Lecomte for preparing the manuscript, Sylvie Salade for plotting the circles, Ruth L. Saul and Janice A. Glaholm for editing, and Professors John B. Southard (Massachusetts Institute of Technology) and Gerald J. Wasserburg (California Institute of Technology) for useful comments.

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Received 20 January; accepted 9 November 1977.

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Palaeolithic–Neolithic seed remains at Franchthi Cave, Greece

FRANCHTHI Cave is located in a limestone headland on the south-west coast of the southern Argolid, Greece. In excavations since 1967 (performed under the direction of Prof. Thomas W. Jacobsen, Indiana University) an almost continuous sequence from the Upper Palaeolithic ~20,000 BC to the Final Neolithic, around 3000 BC, has been uncovered¹. After six excavation seasons, eight trenches have been opened up inside the cave, as well as eight trenches outside the mouth of the cave and along the beach about 50 m below. Owing to the proximity of the deposits to the modern shore line and to the soil surface, no carbonised material has been recovered from these beach deposits. This is unfortunate because the earliest Neolithic on the site has been found in this area. Concentrated water sieving of the trenches inside the cave has recovered large quantities of carbonised botanical material. Most important among these remains has been the discovery of wild oats and wild barley in levels dated to about 10,000 BC, earlier than any botanical remains as yet found in Greece or the Aegean area.

Table 1 Dimensions of *Hordeum spontaneum*

	Length	Breadth	Thickness	L:B	T:B
Tell Mureybit grains*					
Average	5.44	1.89	1.26	290	67
Minimum	3.8	1.5	1.0	252	61
Maximum	6.7	2.1	1.6	372	76
Franchthi Cave grains					
Average	5.69	2.80	1.75	205	63
Minimum	4.2	2.1	1.0	164	47
Maximum	7.5	3.6	2.1	250	90

*After van Zeist and Casparie⁵.

Inside the cave, the two largest and deepest trenches, F/A and H/H-1 quadrants A and B, have been systematically water sieved for the recovery of botanical and other environmental material. Because of the fresh water springs running into Koilada Bay below the mouth of the cave, it was possible to use nearly fresh water rather than salt water for this sieving process. A dam was built and a large hose inserted at the mouth of one of the springs. Water from the spring was then pumped up into two 1 m² storage tanks installed up the slope from the dock on which the smaller sieving tanks were placed. The earth was dumped slowly into these smaller tanks which contained a nylon net mesh of ~ 1.5 mm. The water flowing into the bottom of the tank separated the soil from the rest of the material. The carbonised remains, as well as small snail shells and bones were floated off into another nylon net which was then set on specially built racks indoors to dry.

To date this flot from the south half of F/A and one quadrant of H/H-1 (A) has been sorted, giving a nearly complete sequence from the Upper Palaeolithic (P2233-21,480 ± 350 yr bp; ~ 20,000 bc (all ¹⁴C dates are calibrated with a 5,568 yr half life) through the 'Mesolithic' (a tentative designation for the material from levels dating between 8300 bc to 6000 bc) and Neolithic to the Final Neolithic (P1660-5,261 ± 64 yr bp; ~ 3000 bc). A preliminary identification has been given to most of the botanical remains from this sequence, but measurements have not yet been made on most of the seeds and positive identification for many of the species must wait for further research.

The lowest levels dating from around 20,000 bc up to 11,000 bc contain only species of the Boraginaceae family, namely *Lithospermum arvense* L., *Alkanna* cf. *orientalis* (L.) Boiss., and *Anchusa* sp. A small percentage of these have been burnt, but the majority are in a 'fresh' state. The vast numbers of these species, as many as 2,000 *Alkanna* sp. in one unit, are difficult to explain. There are several possibilities, such as the use of the plant for bedding or fuel, or the use of the root as a dye. Perhaps the seeds were not brought into the cave by man but are the result of rodent or bird activity. There are many bird bones in these levels; this may support the suggestion that these stony seeds were used as 'crop stones' by the birds (S. Payne, personal communication). Further study of the faunal and cultural material from the Upper Palaeolithic may help to solve this problem.

In deposits dated to 11,000 bc lentils were recovered. These are an extremely small species (about 2 mm diameter, but their condition and the present lack of comparative material, will not allow a positive identification to the species level. According to Zohary, both *Lens nigricans* (Bieb.) Godr. (*Ervum nigricans* Bieb.) and *L. ervoides* (Brign.) Grande (*L. lentacula* (Schreb.) Alef.) are small-seeded lentils which are typical Mediterranean elements today. *L. orientalis* (Boiss.) Hand-Mazz. has also been reported from Greece² and is more closely

related to the cultivated *L. culinaris* Medik (*L. esculenta* Moench) than the other two species³. These early lentils at Franchthi may be one of the more common Mediterranean species and are unrelated to the cultivated lentils which appear in the Neolithic deposits on this site. The possibility of their being *L. orientalis*, however, which were collected through the Upper Palaeolithic and Mesolithic and developed into the cultivated variety in the Neolithic must also be considered.

Also occurring in the Palaeolithic deposits at about the same time as the lentils are a species of vetch (*Vicia* sp.). These are small round seeds (2.5-3 mm diameter) which, again, have not been identified to the species level as the hilum is missing from all those otherwise complete enough to measure. The positive identification of the vetches from the cave will be left until it is possible to compare the samples with an adequate modern collection.

At the same time that lentils and vetches begin to appear in the Upper Palaeolithic levels, pistachio (*Pistacia* sp.) and almonds (*Prunus amygdalus* Batsch.) appear. These two species occur together throughout the entire sequence to the Final Neolithic. The *Pistacia* sp. has previously been identified as *P. atlantica* Desf⁴, but the closer examination of the seeds of *P. terebinthus* L. and *P. lentiscus* L. may indicate their presence. Complete measurements of the Franchthi material are needed before any definite species can be assigned. The asymmetry of the Franchthi nutlets suggests that these may be closer to the *lentiscus* than the *atlantica* form.

The almonds have been identified as *Prunus amygdalus* Batsch., but as there is only one whole almond in the entire sequence, it is difficult to be certain of the identification. The whole specimen is 16.5 mm long and 11.8 mm wide. The surface is slightly pitted and grooved and the keel is not prominent.

In levels dated to around 10,500 bc (P1827-12,543 ± 176 yr bp) two grains of oats (*Avena* sp.) and one of barley (*Hordeum* cf. *spontaneum* C. Koch.) have been identified. Neither species occurs again, however, until about 9000 bc in trench H/H-1 A (Palaeolithic) and 7000 bc in trench F/A (Mesolithic), from which points they become major components of the botanical assemblage through the Mesolithic.

There is a problem with the identification of the barley grains in that they are broken and so badly preserved that it is impossible to obtain complete measurements. They have been tentatively identified as *H. cf. spontaneum* on the basis of their similarity to the illustrations of the wild barley reported from the site of Tell Mureybit in Northern Syria⁵, and with modern *H. spontaneum* in the comparative seed collection at the University of Southampton. The grains from Franchthi, however, are larger than those from Mureybit as seen in a comparison of the dimensions (Table 1). Of those grains well enough preserved to measure, it is possible to describe them in much the same terms as used for the Mureybit grain. The dorsal side is longitudinally straight or slightly curved due to puffing during carbonisation. The ventral side is straight or barely convex. In every case one or both ends of the grain is broken off, but they are generally broader at the upper end, tapering toward the lower (radicle) end.

The positive identification of these grains is a crucial factor in the question of early domestication at Franchthi. If the Mesolithic barley is, in fact, a domesticated form it would be the earliest found so far in Greece. Should it prove to be a wild species, however, it raises the question as to whether it is the progenitor of the cultivated barley found in the Neolithic levels at Franchthi. In either case the material will prove to be of great importance in the study of the origins of agriculture in this area.

The oats (*Avena* sp.) will probably not be identified further as there are no lemma bases preserved in the deposits. The grains are very small, the average length being 4.2 mm for the 12 grains so far measured (Table 2). They are spindle shaped, tapering at both ends to a point. On a few of the better preserved grains the hairs are still visible. The number of different species of wild oats in Greece, and the possibility of these being

Table 2 Dimensions of *Avena* sp. from Franchthi Cave

	Length	Breadth	Thickness	L:B	T:B
Average	4.20	1.35	1.13	314	85
Minimum	3.3	1.1	.9	250	64
Maximum	5.5	1.7	1.6	363	145

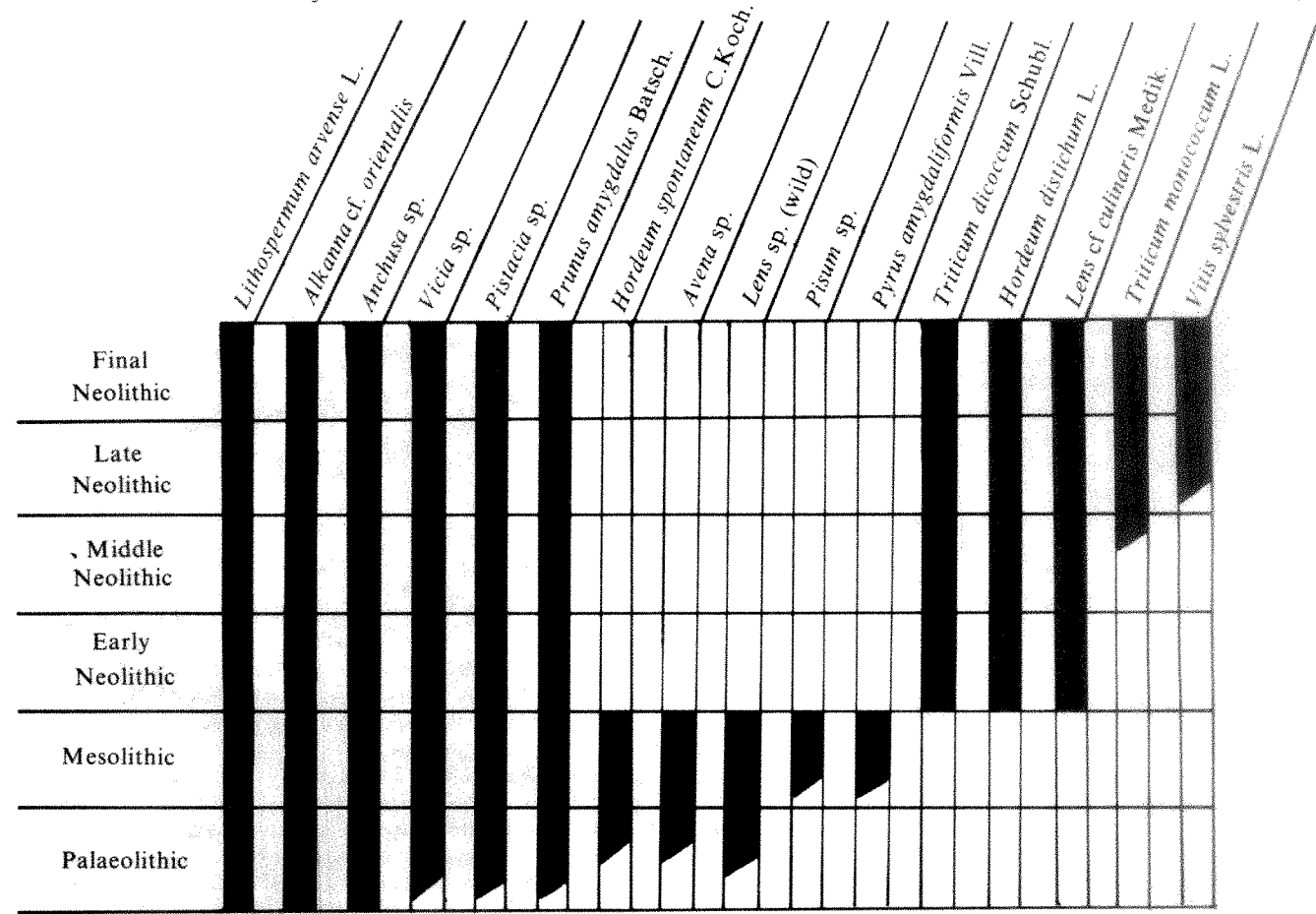


Fig. 1 Botanical remains found in Franchthi Cave.

secondary grains make a definite identification impossible.

Around 8000 bc (P2231–10,260 ± 110 yr bp) in trench F/A the Boraginaceae decrease substantially down to only a few seeds in each unit, and the *Pistacia* sp. increase greatly, becoming the predominant species in the Mesolithic. At one point, however, dated to about 7000 or 6500 bc, there may have been a redeposition of earlier soils as the soil matrix itself is of the reddish, clayey Palaeolithic type and there are vast quantities of *Lithospermum arvense* in these units. At the same time there is a decrease in all other botanical finds in this trench. Further study of the stratigraphy in this area is necessary before we can determine exactly what has happened at this point in the cave's occupation.

Also in the Mesolithic, beginning around 7300 bc (P2230–9,280 ± 110 yr bp), wild pear (*Pyrus amygdaliformis* Vill.) begins to appear. Both whole carbonised fruits and the seeds have been preserved in these very rich deposits. In addition to the lentils and vetches, peas (*Pisum* sp.) have been identified from two Mesolithic levels¹. As yet, no additional finds of this legume have been recovered from these trenches. There are large legumes from these Mesolithic levels which resemble more closely a *Lathyrus* sp., but positive identification must await further study.

The next substantial change in the sequence comes at about 6000 bc (P2095–7,981 ± 105 yr b.p.) with the absence of oats and the first appearance of *Triticum dicoccum* Schubl., cultivated emmer wheat. The change is sudden and complete in trench F/A, occurring through a depth of about 15 cm of earth, and suggests that there may have been a stratigraphic break or a lack of occupation, at least in this part of the cave at this time. *Hordeum distichum* L., cultivated two-row hulled barley, appears shortly after the *Triticum*, both species begin to occur in steadily increasing numbers in levels dated to around

5000 bc. There is also a change in the lentil at this point and those now occurring are the larger, cultivated variety *Lens culinaris* Medik. The vetch also seems to have changed, and the species represented has been tentatively identified as *Vicia ervilia* (L.) Willd. (= *Ervum ervilia* L.). The *Pisum* sp. which had been present in the Mesolithic has not been found in the Neolithic deposits so far examined. Pistachio and almond still occur and very small numbers of Boraginaceae are present in scattered units up to the Final Neolithic.

There is no major change in the species represented throughout the Neolithic with the exception of the appearance of *Triticum monococcum* L. at about 4500 bc in the upper Middle Neolithic levels. This species continues to occur in very small numbers in the late and Final Neolithic as well. Several wild grape pips have been found in the late and Final Neolithic levels and have been attributed to the species *Vitis sylvestris* L.

The extensive water sieving operation (Fig. 1) at Franchthi Cave has therefore, produced, a sequence of botanical material from the Upper Palaeolithic (~ 20,000 bc) to the Final Neolithic (~ 3000 bc). In the Upper Palaeolithic wild species of lentils, vetches, pistachios, almonds, oats and barley were collected. These species remain the primary botanical resources recovered from the cave through the Mesolithic to the Neolithic. In addition, wild pear and peas have been identified from the Mesolithic levels.

There seems to be an abrupt change in these resources around 6000 bc when the wild oats and barley and the wild pear disappear from the botanical material so far examined and the cultivated varieties of emmer wheat, hulled two-row barley, and lentils are found. Cultivated einkorn wheat is present in small amounts from the later Middle Neolithic levels, and wild grape first appears in the Late Neolithic.

The great importance of the Franchthi material is that it

helps to fill a void in our knowledge of pre-Neolithic subsistence in Greece. No other site in this area of the Eastern Mediterranean has yielded botanical material dated to the periods before agriculture had become established. Not only can the Franchthi material answer some of the questions concerning the gathering economy preceding agriculture, but it may also be of primary importance in establishing whether or not agriculture was incipient or introduced to Greece. It has long been felt that the knowledge of cultivation had been brought to Greece from the Near Eastern 'centres'. With the appearance of both earlier, wild forms and later, cultivated forms of certain species at Franchthi Cave, there must be a rethinking of this problem of the origins of agriculture.

Although this is only a preliminary report of the Franchthi botanical material it is hoped that further work will confirm some of the tentative statements and that it will help us to understand the crucial period of subsistence change from plant collecting to plant cultivation.

These excavations were performed under the direction of Professor Thomas W. Jacobsen, Indiana University, as part of the Argolid Exploration Project cosponsored by the University of Pennsylvania and Indiana University¹.

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Received 7 October; accepted 24 November 1977.

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Lipid sacs as a buoyancy adaptation in an Antarctic fish

THE Notothenioidei, the dominant perciform suborder of Antarctic fishes, is a predominantly benthic group of about 75 species¹. They lack a swim bladder, a feature consistent with their mode of life on the bottom of the sea¹. Therefore few of them have been able to exploit the enormous biomass of planktonic crustaceans (krill) indigenous to the mid-waters of the Antarctic Ocean^{2,3}. One notable exception, however, is the nototheniid *Pleuragramma antarcticum* Boulenger which has invaded this highly productive habitat. Unlike other members of this family, *Pleuragramma* spends its life swimming in the water^{2,4}, at times beneath a cover of sea ice. Although *Pleuragramma* lacks a swim bladder, it has been observed to remain nearly motionless in the water when placed in an aquarium. Furthermore, its slow swimming speed and general body morphology (Fig. 1) appear to preclude the use of forward motion and associated hydrodynamic lift as a mechanism for maintaining position in the water column. In -1.8°C seawater, specimens of *Pleuragramma* weighed between 0.5 and 1% of their weight in air indicating they were almost neutrally buoyant. We report here that this reduction in density results from the accumulation of lipid in intermuscular and subcutaneous sacs, and from a reduction in skeletal ossification.

Pleuragramma were collected by lowering a 2-m² net to a depth of 475 m through a 3-m² hole in the ice at McMurdo Sound, Antarctica (77°54'S, 166°40'E). The net was retrieved at the rate of 60 m min⁻¹.

When freshly caught specimens of *Pleuragramma* were trans-illuminated, translucent sacs (0.5–3.0 mm in diameter) containing lipid were seen between the muscle masses at the bases of the dorsal and anal fins. Gross staining of formal-calcium-fixed specimens with oil red O (ref. 5), a stain for neutral lipids, confirmed the presence of lipid and also revealed the existence of many smaller (0.2–1.5 mm) subcutaneous sacs (Fig. 1)

on the sides of the body. Subcutaneous sacs were also seen in the midventral line between the pelvic girdle and the anterior margin of the anal fin. In cross section the intermuscular sacs were medial to the dorsal and ventral-most subdivisions of the epaxial and hypaxial muscle masses (Fig. 2). In this position they were adjacent to the median septa, pterygiophores and the associated erector and depressor muscles of the fins.

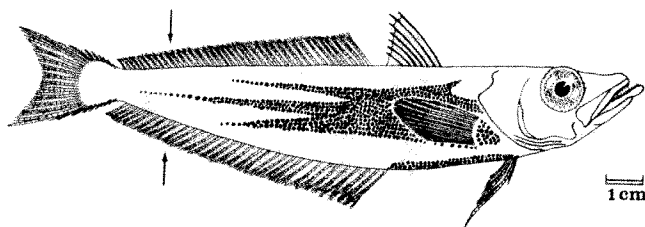


Fig. 1 *Pleuragramma antarcticum* showing distribution of subcutaneous lipid sacs (large black dots) in an adult specimen. Redrawn from Boulenger¹⁵. Arrows indicate the plane of Fig. 2.

Examination of histological sections stained with haematoxylin and eosin or picro-ponceau indicated that the lipid was enclosed in delicate connective tissue sacs, true adipose tissue being rare in fishes⁶.

For analysis, 1 ml of lipid was withdrawn from the intermuscular and subcutaneous sacs of a 30-g specimen. Thin-layer chromatography revealed that the lipid was entirely triglyceride. Extraction and analysis of the total lipid content of the trunk musculature showed that 39% of the dry muscle mass was lipid, with 75% of that being triglyceride (personal communication from P. W. Williams). The methyl esters of the triglyceride fatty acids were prepared⁷ and quantified by gas-liquid chromatography on a column of 10% SP 2340 resin on Chromasorb WAW. The fatty acid composition of the total triglyceride isolated from the muscle was similar to that of the triglyceride present in the sacs.

The density of triglyceride is approximately 0.93 at 0°C. This large amount of extracellular lipid must significantly reduce the density of *Pleuragramma* and would appear to be involved in buoyancy regulation, as is the case with other mid-water fishes which lack swim bladders⁸. The localisation of the lipid in preserved specimens is consistent with this hypothesis; the sacs were largest and most numerous near the centre of gravity of the fish.

Accumulation of lipid was not the only mechanism observed to play a part in density reduction in *Pleuragramma*. Specimens

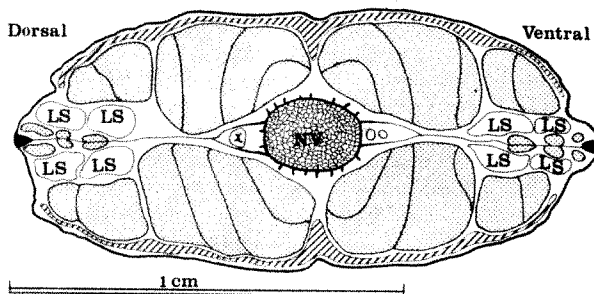


Fig. 2 Cross section of *P. antarcticum* showing location of intermuscular lipid sacs. Hatching, red muscle fibres of lateralis superficialis; stippling, epaxial and hypaxial muscles, erector and depressor muscles of fins; solid black, bone (pterygiophores, vertebral collar and trabeculae); LS, intermuscular lipid sacs; NV, notochordal vesicles (do not contain lipid). Bony tissue in the vertebrae is confined to a thin peripheral collar buttressed by longitudinal trabeculae running the length of the column. These appear as small, lateral projections in this cross section. Notochordal material fills the entire vertebral body and is continuous throughout the extent of the column. The dorsal and ventral median septa connect the tips of the neural and haemal spines to the pterygiophores.

cleared and stained with alizarin red S⁹ had reduced and poorly ossified skeletons compared with other Antarctic nototheniids. For example, the vertebrae were not amphicelous, but merely a thin collar of bone surrounding, and barely constricting, a large core of gelatinous material (Fig. 2), probably representing a persistent notochord. Furthermore, neural and haemal arches and spines were reduced, and ribs were very small. In addition, the scales were thin and weakly mineralised. These observations were further supported by ashing the bones for 10 h at 450 °C. The ashed skeleton of *Pleuragramma* constituted only 0.3–0.5% of the total body weight compared with 0.9% for a closely related but less actively swimming species, *Trematomus borchgrevinki*. In most fishes the ash content of the skeleton is about 2% of the total body weight¹⁰.

It has been suggested before that lipid deposits in liver^{11,12}, bone^{13,14}, integument¹⁴ and muscle¹⁴ play a role in buoyancy regulation. In such cases, however, the precise role of lipids is obscured by the fact that these organs are complicated structures with primary functions unrelated to buoyancy control. In *Pleuragramma* the utility of the lipid sacs in buoyancy regulation can be more easily assessed for two reasons. First, because they are distinct and isolated structures, the only other function attributable to the sacs would be lipid storage before metabolic utilisation. This, however, seems unlikely because the lipid is not in fat cells. Second, there seemed to be an obvious correlation between the appearance of lipid sacs and the degree of skeletal ossification. That is, in small specimens (less than 44 mm) with very slight ossification of the skeleton, subcutaneous and intermuscular lipid sacs were not present. In somewhat larger (45–60 mm) specimens exhibiting some ossification, a moderate number of sacs were present. In large adults showing considerable skeletal ossification, the sacs were extensively developed. The lipid accumulating with increase in size of the fish should serve to offset increasing density resulting from ossification.

Thus it seems that both intermuscular and subcutaneous lipid accumulation and a reduction in skeletal ossification have evolved as specialisations to reduce density in *Pleuragramma*, a pelagic Antarctic fish without a swim bladder. These adaptations enable this fish to maintain the neutral buoyancy necessary for swimming and feeding in the midwaters of the Antarctic Ocean—an ecological niche underutilised by fishes.

We thank Ed Osada for helping net the *Pleuragramma*. This research was supported by the USNSF.

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Equalisation of prey numbers by migratory shorebirds

STUDIES of the effects of predators on prey diversity have usually focused on patterns of predation that do not by themselves increase diversity. For example, size-selective fish decrease diversity by driving large bodied zooplankton to local extinction¹. Size-selective predators can maintain a diversity of species only if large bodied prey hold some advantage over smaller prey, such as the difference in feeding efficiency hypothesised by Brooks and Dodson (ref. 1 and see ref. 2 for review). The diversifying effect of nonselective predation on competitors for space in the rocky intertidal zone³ depends on the presence of differences among competitors in time of settlement during the year. In contrast, selective removal of common prey tends to reverse any numerical advantage gained by one species over another. Thus it is of interest to see whether numerically selective predators increase diversity by equalising prey numbers. The results presented here show that general predators in fact repeatedly equalise the relative abundance of prey species and hence tend to stabilise prey communities.

Migratory shorebirds are flexible predators^{4, 5} that attain considerable numbers in estuaries along the Atlantic coast of North America. Daily counts of shorebirds in the Plymouth, Massachusetts estuary exceeded 5,000 birds from mid-July to mid-August in 1975 and 1976. Most birds belonged to one of four species: sanderling (*Calidrus alba*), semipalmated sandpiper (*Calidrus pusilla*), short-billed dowitcher (*Limnodromus griseus*), and black-bellied plover (*Pluvialis squatarola*).

Each of these species used several foraging techniques at Plymouth, and each showed some degree of habitat selection. Numerical selectivity was evident from examination of crops and gizzards, which generally contained the two or three commonest prey species in the area where the bird had been foraging before collection. Large and abundant invertebrates (*Clymenella torquata*, *Scoloplos robustus*, *Nereis virens*, *N. arenaceodonta*, *Tellina agilis*, *Crangon septemspinosa*, *Acanthohaustorius millsi*, *Trichophoxus epistomus*) were well represented in shorebird diets. Numerical dominants of smaller size (spionid polychaetes, *Gemma gemma*, *Hydrobia (totteni)*, *Protohaustorius deichmannae*) were under-represented relative to their abundance on the flats. Both prey size and prey number seem to be important in the selection of prey by shorebirds⁶.

Invertebrate mortality was estimated by counting the organisms in 20 core samples taken at each of 13 representative sites in July, as birds began increasing in number, and comparing this with the count of organisms at the same sites in September, after most shorebirds had departed. September samples were located within a metre or two of July samples to reduce the effect of a patchy spatial distribution on estimates of invertebrate loss. The sampling procedure itself did not lower invertebrate density, based on the lack of a significant difference between two rounds of sampling at one site on a day in September. All cores were 10 cm in diameter and 10 cm deep. Cores were washed on a 0.5-mm sieve and the organisms visible by eye were counted and recorded in the field. This procedure captures at least 90% of the organisms greater than 2 mm, based on re-examination of samples in the laboratory. The effect of juvenile recruitment on estimates of invertebrate mortality was controlled by recording the size of organisms with sufficient accuracy to distinguish 0-yr class from older animals.

Exclosure experiments in 1975 and 1976 showed that losses between July and September were due to predation. Each exclosure was 1 m on a side, 10 cm high, and made of 14-mm mesh wire. The number of adult invertebrates inside the cages did not change significantly between July and September ($P > 0.50$), compared with the significant declines immediately outside the cages during the same period ($P < 0.05$). Wire cages also exclude horseshoe crabs (*Limulus polyphemus*) and

flatfish (*Pseudopleuronectes americanus*). Flatfish are largely absent from Plymouth Harbour during July and August⁷, when they move offshore into deeper, cooler water⁸. Usage of the study sites by horseshoe crabs did not correspond to invertebrate losses, based on a Spearman rank correlation across the 18 out of 26 0.5-hectare sites where crabs occurred. Mortality at sites used heavily by shorebirds exceeded mortality at less used sites.

Mortality in both years depended on the rank abundance of prey (Table 1). Higher mortality occurred in the initially more abundant prey at a site even though the ranking of individual species in July differed from site to site. Of the numerically dominant macrofauna only *Gemma gemma* did not show substantial losses when abundant. This small (< 4 mm), thick-shelled clam is the commonest species at many sites but it occurs at a low frequency in the gizzards of shorebirds, which suggest that it is relatively unpalatable.

Table 1 Mortality of intertidal invertebrates fed on by migratory shorebirds in Plymouth and Kingston Harbors in 1976

Species rank within sites in July	1	2	3-5	> 5
Average mortality (1-final/initial)	0.84	0.78	0.67	0.36
Average initial density (organisms per 10 cores)	90.9	26.0	26.3	14.1
No. of 0.5 hectare areas	26	26	26	26
No. of different species	6	11	21	32

Mortality is the difference between counts at a site in mid-July and mid-September, divided by the initial count and averaged over sites. All cores were 10 cm in diameter and 10 cm deep. Variation in mortality with frequency was analysed by grouping species according to their rank in July in 10 cores from an area of one-half of a hectare. The change in density within each rank is statistically significant ($P < 0.05$). Changes in mortality between adjacent rankings are all significant at $P < 0.01$, based on a test of the equality of proportions.

Frequency dependent losses resulted in an equalisation of prey numbers during the summer. Equitability ($J' = H'/\ln(s)$, where s is the number of species and H' is the Shannon-Weaver index of diversity⁹), increased significantly from July to September of 1976 ($P < 0.001$), based on a nonparametric Wilcoxon signed ranks test¹⁰. This increase remains significant when the negative association between equitability and collection size has been removed by regression. A similar increase in equitability occurred in 1975. The coefficient of variation¹⁰, another measure of dispersion, decreased significantly in both years.

Shorebirds reduce variation in the relative abundance of their prey by selective removal of numerically dominant species. This would tend to halt the continued expansion of dominant species, since few adult prey escape predation and since these prey reproduce only during the spring and summer. However, differences in palatability make it unlikely that shorebirds could by themselves stabilise an entire community of intertidal invertebrates.

This work was supported in part by contract 14-16-0008-687 from the US Fish and Wildlife Service to the Manomet Bird Observatory. I thank Brian Harrington and the Observatory staff for their help.

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Received 16 September; accepted 28 November 1977.

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Separation of seed development from monocarpic senescence in soybeans

MONOCARPY, a single flowering phase followed by senescence and death, is a widespread phenomenon in seed plants, particularly field crops, whose large monocultures put on a dramatic display during their senescence phase¹⁻⁴. In many, but apparently not all monocarpic species, defruiting or deflowering can prevent or at least delay monocarpic senescence and death¹⁻⁴. For some species such as soybeans, the killing influence has been traced to the developing seeds⁵. The conspicuous correlation between the accumulation of nutrients in the developing fruit and the senescence (or apparent 'exhaustion') of the leaves (Fig. 1) has led to the suggestion that developing fruits cause senescence by diverting or withdrawing needed nutrients or hormones from the leaves and other vegetative parts¹⁻⁴. Here we explore the relationship between nutrient (or hormone) withdrawal or diversion and foliar senescence. Our data indicate that the development (accumulation of dry matter) of the seeds is separable from the senescence response in soybeans, and therefore the seeds may function as more than sinks.

Soybeans (*Glycine max* (L.) Merrill) cv. Anoka were grown in soil as described by Lindoo and Noodén⁶. During the first 4 weeks, the plants were kept in a greenhouse with supplementary light at night and thereafter in environmental control chambers under short (10-h) days. Foliar senescence (yellowing) and fruit development (growth and colour) were measured quantitatively by rapid, non-destructive, visual procedures, which have been checked against the more traditional (but destructive) methods for measuring senescence and fruit development described in detail elsewhere⁶. Fruit development is expressed as fruit maturity index (FMI), which reflects the average state of fruit development based on numerical values 1 (least mature) to 5 (most mature).

Our earlier studies⁶ on soybeans showed the influence of the developing seeds on foliar senescence moves in a restricted pattern within the plant, thereby distinguishing it from the flower-inducing hormone, the movement of which is not as restricted and differs in other ways⁷.

Single axis soybean plants with pods only on the bottom half and foliage only on the top half show greatly reduced foliar senescence compared with unmodified plants and that which occurs is primarily in the leaves nearest to the pods (Fig. 1a). The seed yield of the pod-bearing sections of these plants relative to unmodified plants is not reduced and is, if anything, slightly increased (unpublished data). Subsequent depodding of these 'half-and-half' plants at various stages of fruit development causes an additional reduction in senescence depending on when the pods are removed. Early (up to short day 58) removal of the pods from the bottom half stops foliar senescence, and removal at short day 71 or later does not substantially delay that senescence which does occur.

Figure 1b shows the time course for seed growth (dry weight per plant) and the total seed weight at the depodding. It is important to note that the dry weight of the seeds is nearly maximum (90%) at short day 66 but comparing Fig. 1a with b, it can be seen that depodding, nevertheless, causes an almost total suppression of foliar senescence. These data suggest not only that seed growth can occur without producing foliar senescence, but the senescence is induced mainly during the final phase of seed development when nutrient accumulation is probably complete. The

spatial separation of leaves and pods seems to be important here, for normal plants with leaves subtending the pods do not show this clear distinction between seed growth and senescence when depodded at various stages of seed growth⁵.

When plants are modified to a single leaf and a single pod cluster separated by two nodes, the foliar senescence pattern differs strikingly, depending which is on top, the leaves or the pods (Fig. 2). When the pods are on top, the leaf frequently senesces, whereas it does not when the

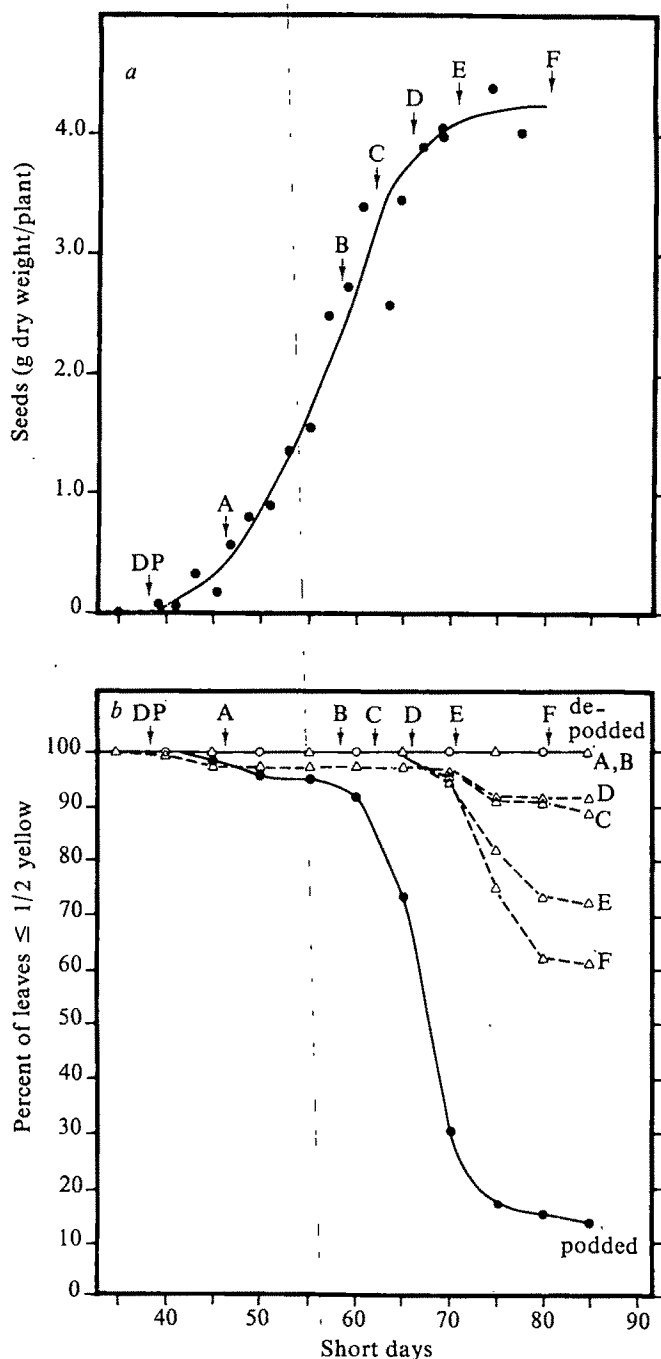


Fig. 1 *a*, Time course of seed growth in surgically modified, single-axis soybean plants (leaves only on top half, pods only on bottom half) and the effect of removing the remaining pods at various stages (indicated by A, B, C, and so on) of seed growth. Surgical modification at FMI 1.4 with subsequent removal of pods as they reached the start of pod fill, stage 2. Measurement of senescence, fruit development and FMI are explained in ref. 6. A single node between the top and bottom halves was both depodded and defoliated. *b*, Time course of foliar senescence in single-axis soybeans with leaves only on the top half and pods only on the bottom half. Same plants as in (*a*). Plants designated 'depodded' were completely depodded at FMI 1.4, whereas the 'podded' plants were not modified at all.

pods are below the leaf. This is consistent with our earlier observation that the senescence signal tends to move downward if at all⁵. Neither delayed fruit development nor reduced seed yield can account for the lack of senescence when the single leaf is on top, the time course of fruit maturation in these modified plants is only 4–10 d behind the unmodified control (podded) plants; the yield per node is actually greater in the plants with a single pod cluster than in the unmodified control (Table 1); and the yield per node is the same whether the pod-bearing node is above or below the leaf (Table 1). Complete defoliation at FMI 1.5 reduces the seed yield in these single pod clusters by 80%; most of that seed growth which does occur apparently occurs before defoliation. The single remaining leaf seems to supply required nutrients for the growing seeds and to be subject to whatever nutrient deprivation is created by the seeds.

The experiments shown in Figs 1 and 2 show in different ways that seed development (and therefore nutrient deprivation) can occur without monocarpic senescence.

The reproductive sink size (seed yield) can be controlled by removing all except a defined number of pods from plants at FMI 1.9 (Fig. 3). In this variety and in the conditions used, seed yield (dry weight) was nearly directly proportional to the number of pods per plant; there was no pronounced tendency for seed size to increase in compensation for a reduced number of seeds. The seed nitrogen per plant is likewise proportional to the number of pods, yet foliar senescence is maximum when the number of pods is only about 40% of the maximum. A direct proportionality between sink size and rate of senescence would be expected if monocarpic senescence were caused by nutrient deprivation; in particular, the saturation of senescence induction at very low pod doses is inconsistent with the nutrient deprivation theories.

The possible explanations for the correlative influence of the developing seeds on foliar senescence fall into four

Fig. 2 Time course of foliar senescence and fruit maturation for single-axis plants pruned to one leaf and one pod cluster (separated by three nodes) compared with unmodified plants and depodded plants with all leaves intact. Depodded and defoliated at FMI 1.5 with subsequent removal of pods as they reached stage 2.

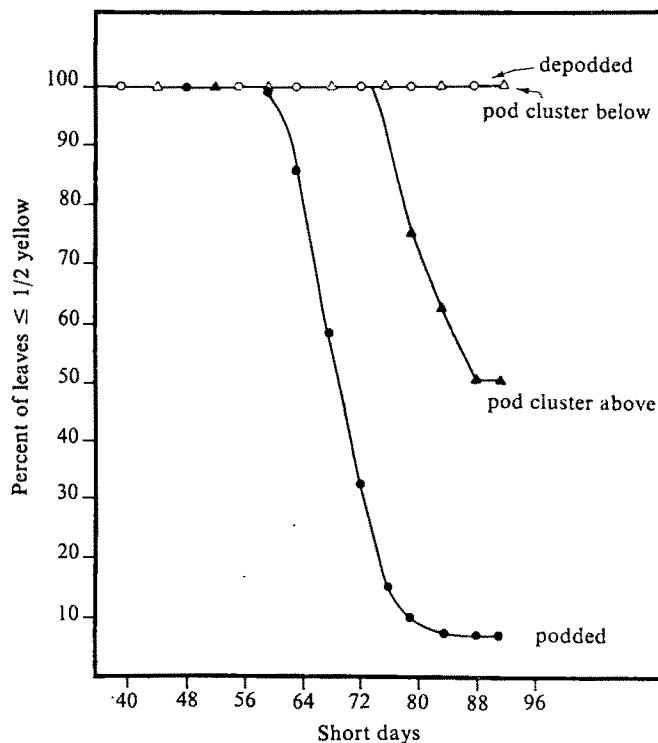


Table 1 Seed yields on single-axis soybeans with only one leaf and one pod cluster* or without modification

	Seeds (g dry weight/node) Unmodified plant	Modified plant*
One pod cluster three nodes above a single leaf (or a pod cluster on a comparable node of an unmodified plant)	1.6	1.5
One pod cluster three nodes below a single leaf (or a pod cluster on a comparable node of an unmodified plant)	0.9	1.9

*Same plants as in Fig. 2. Unmodified plants yield about 8.3 g of dry seeds each.

general categories, which have some variations and combinations⁴ but can now be evaluated in the light of the data for soybeans. The first, and least precisely defined, holds that the plants simply have a limited lifespan which runs out about the time the seeds develop. One variant of this hypothesis invokes a limited lifespan for the meristems, the loss of which causes death. Although it may be true that the shoot apical meristems degenerate during flower development thereby limiting vegetative growth (this point needs study in soybeans), depodding of soybeans at an early stage can prolong the life of these plants way beyond normal, possibly indefinitely, even though they undergo little or no vegetative growth. Thus the senescence and death we have observed in conditions favourable for soybean growth is coupled to seed production and not time or meristem degeneration.

The second hypothesis has the developing fruit diverting nutrients (for example, minerals from the roots, photosynthetic assimilate from the leaves) or hormones (cytokinins from the roots) away from the leaves thereby causing their death. Diversion of photosynthetic assimilate from the leaves seems an unlikely cause, for mature or senescent leaves seem to produce more than they need^{10,11}. The diversion hypothesis, particularly the diversion of substances from the roots, is difficult to reconcile with the limited

movement of the senescence 'signal' from the seeds¹. If such a diversion were important, then why does the single pod cluster cause senescence of the single feeder leaf only when the feeder leaf is below the pods and not when above? One would expect the pods to divert substances from the roots more effectively when they are below the leaf. Furthermore, the idea that foliar senescence is triggered by a reduction in the supply of substances produced by the roots, is also countered by the observation that foliar senescence occurs in these plants where the leaf- or pod-to-root ratio is greatly reduced. In addition, the delayed depodding of single-axis plants with pods only on the bottom half and leaves only on the top half suggests that senescence induction by the seeds may occur after most of the seed growth and its concomitant nutrient demands are complete. Note that in order to prolong the life of the plants and to maintain their productivity, expert gardeners take care to remove the fruits from certain varieties of cucumbers and beans after they fill out but before their colour changes (for example, after drain or diversion is completed but before monocarpic senescence is initiated). Finally, the sink size (pod dose or seed yield) does not parallel foliar senescence; the senescence response is saturated at a level far below the maximum level of dry weight and nitrogen accumulation in the seeds.

The third hypothesis involves nutrient drain; the developing fruit are supposed to withdraw nutrients from the leaf to the point of killing them. Indeed, there probably is some exodus of certain nutrients (particularly nitrogen) from senescing leaves¹. The arguments against diversion, however, apply equally well to drain.

The fourth explanation gives the developing fruit or seeds a more active role: production of a substance(s) which travels out to the leaves to cause senescence and ultimately the death of the whole plant.

Although drain and diversion may be involved in the monocarpic senescence of soybeans, it seems unlikely that developing seeds exert such remarkable correlative influences simply by functioning as passive sinks, just as shoot apices do not exert apical dominance solely through nutrient diversion. Such a hormone(s) will, of course, remain hypothetical unless actually isolated and shown to travel from the source (the seeds) to the target (leaves and other parts).

Since monocarpic senescence must have evolved independently in a wide range of groups, it may well be caused by different mechanisms in other species. Indeed, the flowers of male spinach induce monocarpic senescence, even though they do not produce seed². Deflowering retards this senescence, and neither nutrient drain nor diversion seem to be causal. On the other hand, it has been reported that defoliation of cocklebur does not even delay the senescence and death of the whole plant¹².

This study was supported in part by a research grant (416-15-79) from the USDA Cooperative State Research Service under P.L. 98-106. We thank Rohbe Crotz for help in growing the plants during their first 8 weeks and Gary Kahanak for help in completing one set of experiments.

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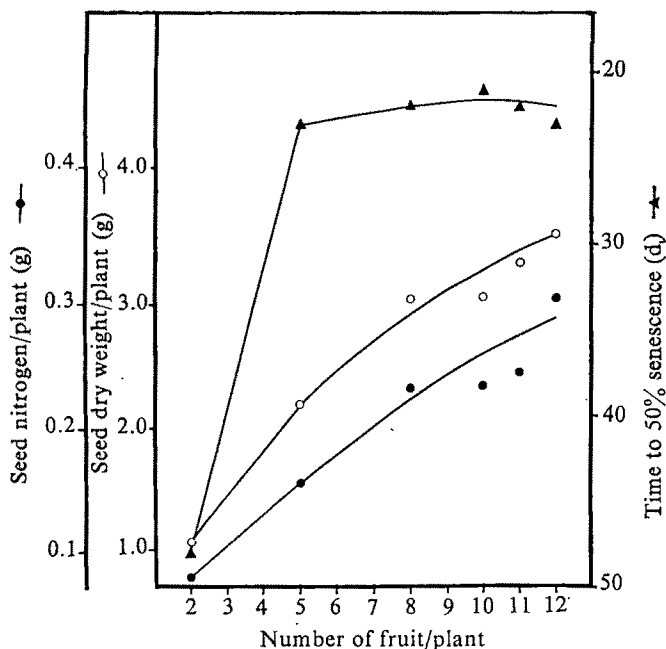
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Fig. 3 Relationship between sink size (pod number) and rate of foliar senescence. The pods were pruned to the designated number at FMI 1.9, and thereafter extras were removed as they reached stage 2. The average number of pods on unmodified plants was 14. Total nitrogen was measured by digesting samples at 280 °C in concentrated H₂SO₄ containing CuSeO₄ catalyst followed by dilution with H₂O and addition of Nessler's reagent for colorimetric determination^{8,9}.



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Regulation of RNA synthesis in early germination of isolated wheat (*Triticum aestivum* L.) embryo

THE change from dormancy to germination of seeds requires water and an appropriate temperature. Wheat embryo germination is characterised by an initial increase in fresh weight, followed by a 5-h lag¹. Although RNA synthesis is one of the earliest biologically measurable activities in wheat embryo germination^{2,3}, its mode of regulation has not been established. Reports^{3–6} have suggested that the rate of synthesis does not change during the first 6 h of germination. But no adjustment was made for the specific activities of ribonucleoside triphosphate, UTP. This would have led to a misinterpretation of incorporation data when there was either a change in the concentration of the nucleotide or a difference in the rate of phosphorylation from nucleoside to nucleotide as germination progressed. To investigate further whether there is a change in the rate of RNA synthesis in the germination phase, we have used recently-developed enzyme assays^{7–9} to measure picomole changes in purine pyrimidine ribonucleotide levels. We found a threefold increase in the rate of RNA synthesis of wheat embryos germinated for 40 min–5.5 h. We used the reaction catalysed by uridine 5'-diphosphoglucose pyrophosphorylase to determine the content of UTP. The key step in this analysis is the selective adsorption of the reaction product, UDP-¹⁴C-glucose, on to activated charcoal in the presence of 0.8 M Trizma base⁸.

In a 15-min pulse-labelling experiment, a 5.1-fold increase in the rate of ³H-uridine incorporation into RNA was observed in wheat embryos germinated for 40 min–5.5 h (Table 1, experiment 1). When incorporation data were corrected by adjusting for the specific activity of the UTP, there was a 2.6-fold increase in ³H-uridine incorporation into RNA during the germination phase. When germinating wheat embryos were pulse-labelled for 15 min longer followed by RNA isolation and purification, there was a 2.9-fold increase in 5.5 h (Table 1, experiment 2), similar to the 2.6-fold increase of experiment 1. These results show that the rate of RNA synthesis increases threefold during the period of germination between 40 min and 5.5 h. These results are in contrast to the idea that RNA synthesis does not change during the first 6 h of germination^{3–6}.

The rate of RNA synthesis in the early stage of the germination phase could be limited by either (1) RNA polymerase activity; (2) changes in the template activity of chromatin, or (3) changes in purine and pyrimidine ribonucleoside triphosphate levels. Possibilities (1) and (2) are less likely because the extraction and assay of RNA polymerase does not change for the first 6 h¹¹, and Yoshida and Sasaki¹² reported that the template activity of chromatin is essentially constant for the first 18 h of germination.

There is the following support for possibility (3). To explain the difference in RNA-synthesising capacity between embryos germinated for 40 min and those germinated for 5.5 h, the concentrations of purine and pyrimidine ribonucleoside triphosphates were measured. We found that the cellular level of ATP and GTP increased 60% and 30%, respectively. This minor increase in purine ribonucleoside triphosphates does not corroborate the threefold increase in the rate of RNA synthesis during this period. However, when the pyrimidine ribonucleotide pool was assayed, there was a 400% increase in the level of pyrimidine ribonucleotides during the period between 40 min and 5.5 h of germination (Table 2). The increase of UTP was similar to that of CTP. Thus this large increase in the substrate levels of UTP and CTP could account for

Table 1 Rates of RNA synthesis in wheat embryo during the germination phase

Experiment 1	Labelling periods	
	40–55 min	5.5–5.75 h
5% TCA-insoluble radioactivity	4,130 ± 176 c.p.m.	20,900 ± 377 c.p.m.
Specific activity of UTP	(1)	(5.1)
Adjusted incorporation	1,788 ± 71 c.p.m. nmol ⁻¹	3,511 ± 211 c.p.m. nmol ⁻¹
	(1)	(2.6)
Experiment 2	30–60 min	5.25–5.75 h
RNA fraction	20,410 ± 1,160 c.p.m.	115,040 ± 5,640 c.p.m.
Adjusted incorporation	(1)	(5.6)
	(1)	(2.9)

To prepare the trichloroacetic acid (TCA)—insoluble extract, isolated wheat (*Triticum aestivum* L. variety Fortuna) embryos (50 mg) were germinated in a Petri dish (60 × 15 cm) on four layers of 4.25-cm diameter Whatman No. 1 filter paper with 2 ml of water (containing chloramphenicol, 20 µg ml⁻¹) in the dark at 25 °C for 40 min or 5.5 h. The top layer containing the embryos was removed, blotted and placed in 0.5 ml of water containing chloramphenicol (20 µg ml⁻¹) and 50 µCi of purified 5-³H-uridine (25 Ci mmol⁻¹) for 15 min at 25 °C. The paper was blotted and the embryos were rinsed with water. Embryos were ground first with 0.5 ml of 10% TCA and then with 5 ml of 5% TCA. The homogenate was centrifuged for 10 min at 23,500g and the supernatant was kept. The pellet was rinsed three times by suspending in 5 ml of 5% TCA with a motor-driven Teflon homogeniser and centrifuging for 5 min at 23,500g. The pellet was then suspended in 1 ml of 0.1 N NaOH and counted in 10 ml of Triton X-100 toluene-based scintillation fluid. The TCA-soluble extract was prepared as before⁸. Unlabelled UTP (1 µmol) was added to the extract and concentrated down to dryness at 40 °C in a flash evaporator. A quarter of the sample was spotted on a PEI-cellulose TLC (Brinkmann) which was then developed in either 0.5 M KH₂PO₄, pH 4.5, or 0.4 M LiCl, pH 5.5. The UTP region on the PEI-cellulose was removed from the TLC sheet by treating with 1 ml of 2 M LiCl. The suspension was then mixed with 10 ml of liquid scintillation fluid (ACSTM, Amersham/Searle). The endogenous UTP was determined as before¹⁴. To prepare the RNA fraction, 50 mg of isolated wheat embryos was germinated as before and labelled for 30 min with 70 µCi of 5-³H-uridine. The embryos were ground first with 0.5 ml of high salt buffer (1 M KCl, 100 mM Tris-Cl, pH 7.6, and 5 mM EDTA). Sodium dodecyl sulphate buffer (3 ml of 1% SDS, 10 mM Tris-HCl, pH 7.6, and 5 mM EDTA) was added and the mixture was vortexed for 2 min. It was then extracted with water-saturated chloroform: phenol (1:1, v/v). The aqueous fractions were combined, mixed with two volumes of absolute ethyl alcohol and stored overnight at -20 °C. The precipitate was centrifuged at 23,500g for 20 min, rinsed three times with 5 ml of cold absolute ethyl alcohol, and then dissolved in 1 ml of 0.5% SDS buffer (0.5 M NaCl, 10 mM Tris-HCl, pH 7.6, 0.5% SDS and 1 mM EDTA). Sample of 40 µl was mixed with 10 ml of liquid scintillation fluid (ACSTM) and counted as before. The data represent an average of duplicate samples. Numbers in parentheses signify the fold increase of the 5.5-h values above those of the 40-min values.

Table 2 Cellular levels of nucleoside triphosphates in early germination of wheat embryos

Germination time	ATP	GTP pmol per mg of embryo	UTP	CTP
0	8	46	<1	<1
40 min	1,288 (1)	320 (1)	132 (1)	84 (1)
3 h	2,000 (1.6)	304 (1)	420 (3.2)	200 (2.4)
5.5 h	2,072 (1.6)	425 (1.3)	540 (4.1)	320 (3.8)

Adenine nucleotides were analysed by the method of Cheung and Marcus⁷. The key reaction is the phosphorylation of ADP by ³²P-PEP in a reaction catalysed by pyruvate kinase, with the extent of transfer of ³²P to ADP determined by adsorbing the nucleotides on to charcoal. ATP is determined as ADP after being converted quantitatively by hexokinase in the presence of excess glucose. Guanine ribonucleotides were determined according to Cheung and Marcus⁸. The principle of the assay is the conversion of GDP to ³²P-GTP utilising ³²P-PEP in the pyruvate kinase reaction. ³²P-ATP which is also formed as a major product is removed with glucose and hexokinase. CTP was determined with RNA polymerase^{13,14}. A reaction mixture in a volume of 100 µl, containing 12 mM MgCl₂, 80 mM KCl, 60 mM Tris-HCl, pH 7.6, Triton X-100 (0.02%), 0.9 U of *E. coli* RNA polymerase, 17.5 µg of calf thymus DNA, 50 nmol of GTP, 50 nmol of UTP, 2 nmol of ATP, 2.5 µCi of 8-³H-ATP (17 Ci/mmol⁻¹), and 50–200 pmol of CTP was incubated for 1 h at 37 °C. The suspension was then diluted with 5 ml of cold 5% TCA and filtered through glass fibre filters (Whatman GF/C). The filters were washed with 5 ml of 5% TCA, three times, dried and counted in 10 ml of toluene based scintillation solution. Figures in parentheses are as in Table 1.

the increase in RNA-synthesising capacity in the late germination phase.

Although the template activity of chromatin is essentially constant for the first 18 h of germination and active RNA polymerase is already present in the resting embryo, the amount of ribonucleoside triphosphates present in the resting embryo is almost negligible (Table 2). On the basis of these observations, we conclude that the rapid increase in the cellular content of purine and pyrimidine ribonucleoside triphosphates triggers the initial RNA synthesis once the wheat embryo is exposed to germination conditions.

We therefore propose that there are at least two stages of RNA synthesis in the early germination of wheat embryos. In the first stage (from 0 to 40 min), there is an increase in the four ribonucleoside triphosphates. In the second stage (from 40 min to 5.5 h), only the pyrimidine nucleoside triphosphate levels increase significantly. Thus the synthesis of RNA initially depends on the presence of all four ribonucleoside triphosphates. This is followed by a stage in which the pyrimidine ribonucleoside triphosphates determine the extent of RNA-synthesising capacity.

The mode of regulation of RNA synthesis in early germination of wheat embryo could be used as a working model for other biological systems. Changes in purine or pyrimidine pools may be used to explain the data of Hentschel and Tata¹⁵. They observed a differential activation of free and template-engaged RNA polymerase I and II during the resumption of development of dormant *Artemia gastrulae*. The level of uridine triphosphate has also been shown to control the growth in ascites hepatoma cells¹⁶.

We thank Dr Abraham Marcus for wheat embryos. This research was supported by a grant from the NSF.

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Received 28 June; accepted 30 November 1977.

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Response properties of dipteran giant visual interneurons involved in control of optomotor behaviour

GIANT homolateral interneurons in the third optic ganglion of flies respond to visual stimulation with graded potentials only, lacking spike generation for signal transmission in physiological conditions¹. (The term 'homolateral' is used here in its anatomical connotation of neurones which are anatomically restricted to one side of the brain⁵.) Graded potentials were thought to be the means of signal transmission of all three classes of homolateral giant elements of this neuropile region, that is, the 'vertical cells' (V cells), the 'centrifugal horizontal cells' (CH cells) and the 'horizontal cells' (H cells): both CH and H cells are mainly sensitive to horizontal pattern movement, whereas the V cells respond mainly to vertically moving patterns^{1,2}. (The CH cells have an additional sensitivity to vertical, some V cells to horizontal pattern motion.) However, there is experimental proof that these findings might be valid only for the CH cells in which exclusively graded potentials could be recorded showing discrete excitatory and inhibitory postsynaptic potentials^{1,2}. For the V cells, however, it has been shown that spiking can be induced by application of hyperpolarising currents². In the absence of hyperpolarising currents, graded potentials with superimposed 'local' action potentials of small amplitude were observed^{1,2}. The H cells, on the other hand were reported to generate only 'local' action potentials superimposed on to postsynaptic slow potentials¹: these findings¹ are not compatible with the previous³ results or with the results presented here which demonstrate regular action potential activity in H cells in physiological conditions.

Intracellular recordings were obtained from various sites along the axons of horizontal cells. The site of penetration was not marked but could be estimated with high accuracy by using external landmarks of the neuropile for reference—for example tracheal patterns and borders of the ganglia. After obtaining the recordings, cells were marked by iontophoretic injection of the fluorescent dye Procion yellow M4RAN so that individual cells could be identified anatomically.

Figure 1 shows two of the three types of horizontal cells, one north cell (NH) and two equatorial cells (EH) following the terminology of Pierantoni⁴. Note that even the very fine dendritic processes are completely stained as comparisons with cells stained by other methods show^{5,6}.

Figure 2 shows intracellular recordings from the three cells shown in Fig. 1. In all three cases, the stimulus consisted of a moving pattern projected on to a circular milky glass screen. This moving pattern stimulated either the contralateral or the ipsilateral eye—the H cells have a binocular sensitivity—subtending a viewing angle of approximately 70° at the fly's eye. For all three recordings shown, the direction of pattern motion was such that the H cells reacted with excitatory responses. Figure 2a shows a d.c. recording obtained from the axon close to the dendritic tree in the lobula plate (see arrow), cell EH1. The cell's response was elicited by ipsilateral stimulation with a progressively moving pattern (that is, movement from the anterior to the posterior). A similar response can be obtained by recording from the dendritic branches in the lobula plate. It is clearly seen that strong excitatory and inhibitory postsynaptic potentials are superimposed, revealed by the steep depolarising

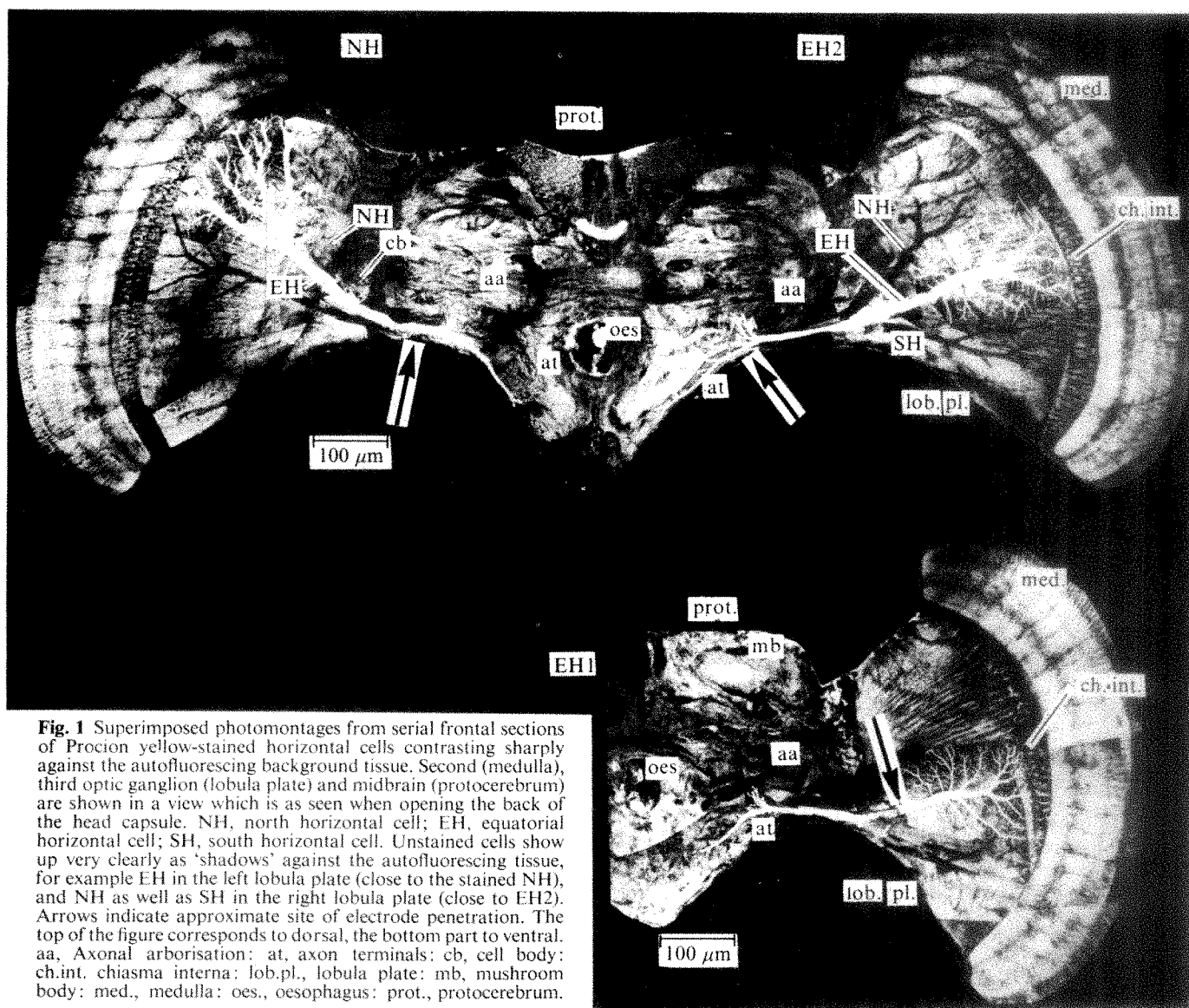


Fig. 1 Superimposed photomontages from serial frontal sections of Procion yellow-stained horizontal cells contrasting sharply against the autofluorescing background tissue. Second (medulla), third optic ganglion (lobula plate) and midbrain (protocerebrum) are shown in a view which is as seen when opening the back of the head capsule. NH, north horizontal cell; EH, equatorial horizontal cell; SH, south horizontal cell. Unstained cells show up very clearly as 'shadows' against the autofluorescing tissue, for example EH in the left lobula plate (close to the stained NH), and NH as well as SH in the right lobula plate (close to EH2). Arrows indicate approximate site of electrode penetration. The top of the figure corresponds to dorsal, the bottom part to ventral. aa, Axonal arborisation; at, axon terminals; cb, cell body; ch.int. chiasma interna; lob.pl., lobula plate; mb, mushroom body; med., medulla; oes., oesophagus; prot., protocerebrum.

and hyperpolarising potential flanks, respectively. In addition, a depolarising average membrane potential shift is observed. Figure 2b shows a recording from the axon of NH closer to the protocerebrum (in the optic peduncle connecting the lobula plate with the protocerebrum), that is, the recording was obtained close to the axonal arborisation (aa in Fig. 1) in the posterior slope. Presenting regressively moving patterns to the contralateral eye, very distinct excitatory postsynaptic potentials and rapid 'spike-like' potentials (the 'local action potentials' of Hausen¹) can be observed. For very effective stimuli, these spike-like potentials become superimposed. The ability of the axon to sum these spike-like potentials has been considered to be caused by a passively conducting membrane^{1,2}. In other words, the response shown in Fig. 2a and b can be obtained at any location along the axon. This is easily understood by calculating the space constant of such an axon which yields $\lambda = 1,000 \mu\text{m}$ (refs 1,2).

However, Fig. 2c shows a recording from a site proximal to the axonal arborisation (aa, Fig. 1). Again a regressively moving pattern was presented to the contralateral eye, using the same stimulating condition as for the experiments shown in Fig. 2b. It can be clearly seen that the cell is capable of generating regular regenerative action potentials; no slow membrane potentials accompany the action potentials. A refractory period was always detected—that is, we never observed 'superimposed spikes'. These results were not found to be cell specific, but representative for all H cells.

With high probability we can exclude the possibility that these findings (particularly the spiking activity of the axonal terminals of the H cells or the graduated potentials of the axon connecting lobula plate and protocerebrum) are injury potentials due to microelectrode penetration or to other unnatural conditions of the preparation. No changes in response properties were observed even after up to 80 min of intracellular recording time, and high frequency firing rates were not seen when cells were damaged by penetration. Furthermore, typical cell responses could be obtained from other cells even up to 2 h after obtaining recordings such as those shown in Fig. 2. However, definite proof would require the demonstration that the animal is capable of, for example, optomotor responses after penetration of such a cell.

These findings are consistent with the concept (to be fully discussed elsewhere (H.E.K., in preparation)) that (1) spikes are generated in the axon part between the axonal arborisation (aa) and the axonal terminals (at) and thus the 'local spikes' reflect the spike activity of the terminal axon part since these spikes are conducted retrogradely in the passive axon membrane distal to the axonal arborisation. (2) In addition to the ipsilateral input in the dendritic fan of the lobula plate, there is a second synaptic input region, which is most likely situated at the axonal arborisation and/or the axon terminals, and which is made by contralateral elements (see also ref. 1).

Thus the giant axon connecting the dendritic fan of the lobula plate with the input region in the protocerebrum could be used

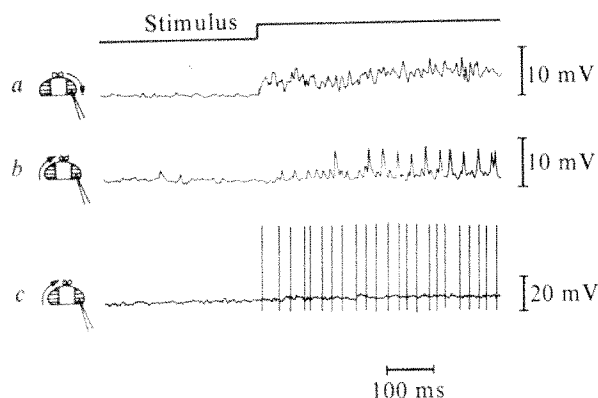


Fig. 2 Intracellular recordings obtained from the H cells shown in Fig. 1 show responses to ipsilaterally presented progressive (a) and to contralaterally presented regressive pattern motion (b,c). Pattern parameters were: spatial wavelength, 20° ; contrast, $m = 48\%$; velocity, $w = 5 \text{ deg s}^{-1}$, luminance 4 cd m^{-2} . Arrows at the schematic horizontal cross section of the head (to the left of the response traces) indicate the direction of pattern movement.

for integrating ipsilateral as well as contralateral inputs. It is not clear whether the spike activity shown by these fibres represents the output of these cells or the contralateral input, but the former seems to be the more plausible interpretation³.

This work was supported by the Deutsche Forschungsgemeinschaft. Part of the work was carried out at the University of Southern California Department of Biological Sciences, Los Angeles, and supported by grants from the NSF (BMS 74-21712) and NIH (1 ROL EY 01513-01).

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Directed outgrowth of optic fibres regenerating *in vitro*

THE way in which growing nerve fibres find their destined sites of termination is largely unknown, although there are supporting arguments and documentation for both local and long-range guidance mechanisms¹⁻³. Fibres regenerating following axotomy tend to grow in the direction of the degenerating tracts, apparently guided through channels formed by debris and/or glial cells^{4,5}. Here we present evidence that the neuritic outgrowth from goldfish retinal explants is related to the orientation of cut fibres within the explant.

We demonstrated previously that optic nerve crush 10-14 d before retinal explantation will stimulate neurite production in culture⁶ and that the neurites originate from ganglion cells⁷. On the day of explantation the retina is cut into square pieces $500 \mu\text{m}$ across, which are placed on a poly-L-lysine-coated substratum⁸. Several explants are placed in each 35-mm dish (NUNC) without regard to whether it is the vitreal or the photoreceptor surface that contacts the substratum. The ganglion cell axons in these experiments have been cut twice: once, at the time of optic nerve crush, several millimetres from the ganglion cell soma; and again, close to the soma when the retina is cut for explantation.

Examination of explant cultures under phase microscopy indicated that neuritic outgrowth began within a day of explantation. The direction of outgrowth was influenced by several factors. For example, neurites grown on poly-L-lysine-coated planar surfaces (glass or plastic) inevitably curved in a clockwise direction. Experiments designed to reveal the nature of this tendency led us to the conclusion that the directionality reflects an inherent helicity of the fibres⁹.

The clockwise directionality of neuritic growth is apparent in Fig. 1, which also indicates another property of the explants: the early outgrowth was usually restricted to one side or corner (Fig. 1a). A probable explanation for this asymmetric outgrowth was provided by explants stained with Holmes silver nitrate¹⁰. An array of parallel fibres could be seen in the optic fibre layer (Fig. 2), and the region of densest neuritic outgrowth was often aligned with this axis. Since the optic fibres in the goldfish retina, as in other vertebrates¹¹, are arranged in straight, radial lines, all of which converge at the optic disk where the fibres exit the eye as the optic nerve, it seemed probable that the parallel fibres within the explant were the ganglion cell axon tracts. Within the small area of the explant, convergence of the fibres was not apparent. Thus, it was not possible to determine which was the centripetal end of the fibre axis, so we could not establish whether the outgrowing fibres were indeed pointing at the phantom optic disk. In a brief, undocumented report 30 years ago, Vinnikov described directed outgrowth from retinal explants of several vertebrate species which he attributed to the radial pattern of optic fibres¹².

In order to establish definitively whether the neurites were aligned with the pre-existing ganglion cell fibre tracts within the explant, we performed the experiment represented schematically in Fig. 3. A strip of retina approximately $750 \mu\text{m}$ wide, extending across the full expanse of the retina from one peripheral margin to the other and including the optic disk, was prepared and then cut into thirds. Unlike our usual explants,

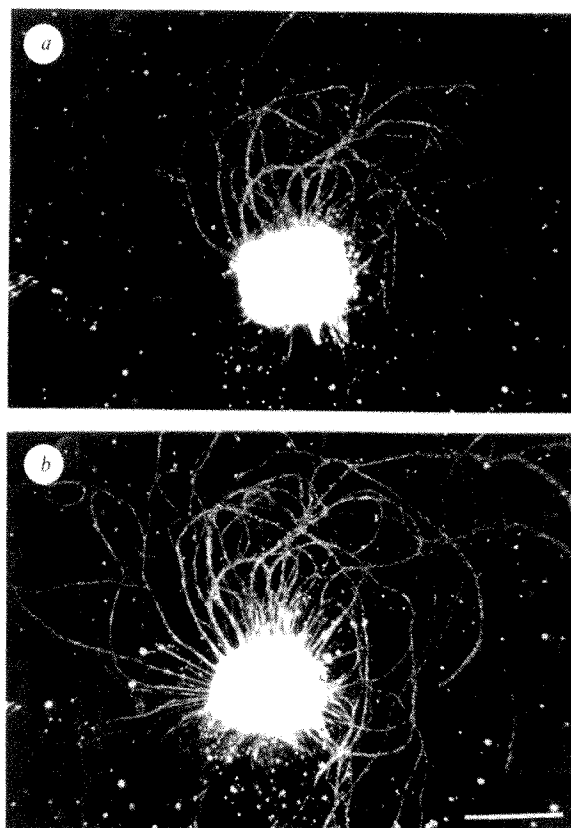


Fig. 1 Retinal explant viewed with darkfield illumination. The majority of neurites were restricted to one edge after 4 d *in vitro* (a). Note that most of them curve in a clockwise direction. After 14 d (b), neurites had grown out from the other edges as well. Scale bar, $500 \mu\text{m}$.

the orientation of each of these pieces was known. We expected no growth from the edges corresponding to the peripheral margins of the retina and very little from the two lengthwise edges, since the latter intersected few of the 'spokes' formed by ganglion cell axons. Further, we anticipated little growth from the central explant, since its ganglion cell axons would be aimed inward. The experimental results shown in Fig. 4 indicate that the neurites grew in the predicted direction. The pattern of outgrowth shown was observed in several replications and was independent of the placement of strips with respect to one another in the dish. The observed asymmetry of early outgrowth was thus a property of each piece of retinal tissue and did not reflect interaction between explants or other extrinsic factors.

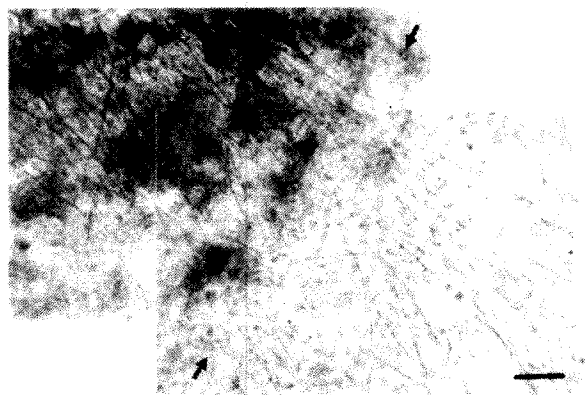


Fig. 2 In this silver-stained explant (6 d *in vitro*), the optic fibre layer can be seen at the upper left; the edge of the explant lies between the two arrows. Neurites aligned or contiguous with fibres in the explant have grown out on to the substratum at the lower right. Scale bar, 25 μ m.

After several days in culture, the asymmetry of outgrowth was diminished as fibres emerged from the entire circumference of both the small, square explants (Fig. 1b; see also previous studies^{7,9}) and from the large strips. Examination of silver-stained explants offered a possible explanation for the eventual appearance of outgrowth from edges which were initially bare. Many of the later-appearing fibres had wandered through the explant or were deflected at the tissue-substratum boundary and grew along the edge of the explant for some distance before emerging on to the dish. In contrast, the first neurites seen probably regenerated from axonal stumps whose cut ends were lined up along the edge of the explant nearest the optic disk, so that their path was unimpeded as they grew out on to the substratum.

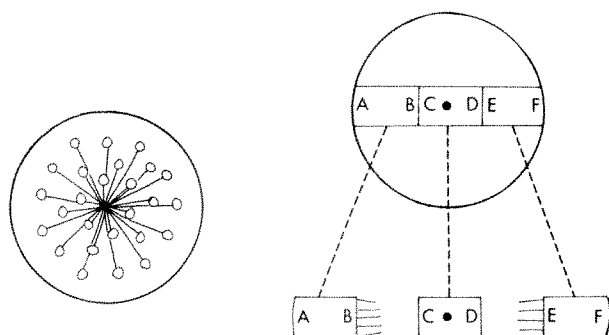


Fig. 3 The schematic diagram on the left shows the radial pattern of optic fibres in the retina. Ganglion cell somata are represented by small, open circles and their axons by line segments which converge at the optic disk (filled circle). At the upper right, the retina is shown as it was prepared for experimentation. A strip of retina that included the optic disk was divided longitudinally into three approximately equal pieces and then explanted. If the neurites maintained their centripetal orientation (towards the disk), they would grow out, as shown at the bottom right, from the ends labelled B and E.

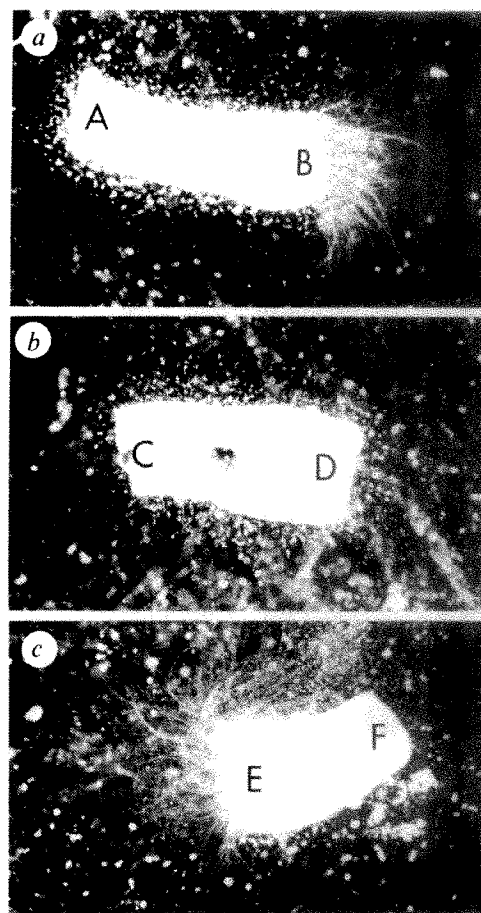


Fig. 4 The results of an experiment as described in Fig. 3 are illustrated. After 6 d *in vitro*, most of the neurites have grown out as predicted from ends B (a) and E (c). The optic disk appears as a dark spot in b. Scale bar, 500 μ m.

The explants in Fig. 4 each contain approximately 5,000 ganglion cells¹³ from which axons regenerated in a direction predictable from their pre-existing pathways. While it is well known that growing axons can be guided both *in vivo* and *in vitro*^{1,14,15}, in these retinal explants a great many fibres have been channelled in the same direction in the absence of extrinsic influences. This preparation thus seems to provide a unique opportunity to directly examine retinal fibre-fibre interactions and their proposed role in neuronal recognition^{16,17}.

We thank Dr Anne M. Heacock for useful suggestions. This study was supported by NSF grants BMS 75-03810 and NIH MH 12506-10 to B.W.A. P.R.J. was supported by NIH postdoctoral fellowship NS 05518-01.

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Neuromuscular transmission is adequate in identified abnormal dystrophic muscle fibres

It has been suggested that 'functional denervation', a failure of structurally intact neuromuscular junctions to evoke a muscle fibre action potential following nerve stimulation, plays an important part in the pathogenesis of murine muscular dystrophy^{1,2}. Other workers, however,

have been unable to confirm the existence of 'denervated' muscle fibres in dystrophic mouse muscle³. Moreover, the demonstration that transmitter release in response to nerve stimulation is normal in such muscle⁴ seems to preclude the possibility that 'denervation' may arise in an unpredictable or spasmodic way as a result of changes in transmitter store size or mobilisation during or after the repetitive discharge of the motoneurone. It has been considered possible, however, that the microelectrode techniques used in many of these studies do not always sample diseased muscle fibres, but select only a hypothetical group of 'normal' or 'healthy' muscle fibres. We have therefore applied a technique of intracellular staining^{5,6} and demonstrated that structurally abnormal muscle fibres in dystrophic muscle display normal neuromuscular transmission.

Experiments were carried out at room temperature on extensor digitorum longus (EDL) muscles removed from 3-6-months-old dystrophic (Bar Harbor 129 ReJ) mice and their clinically normal litter mates. In some muscles, muscle fibre action potentials were generated in response to indirect excitation and were recorded using standard intracellular techniques. In other muscles, endplate potentials (e.p.s) were recorded following nerve stimulation at frequencies of 3 Hz and 30 Hz in the presence of 0.6-1.2 μ M *d*-tubocurarine. The quantum contents of the e.p.s were estimated from the coefficient of variations of e.p.p. amplitudes⁷. After recording either action potentials or e.p.s, a second micro-electrode filled with a 4% (w/v) aqueous solution of Procion

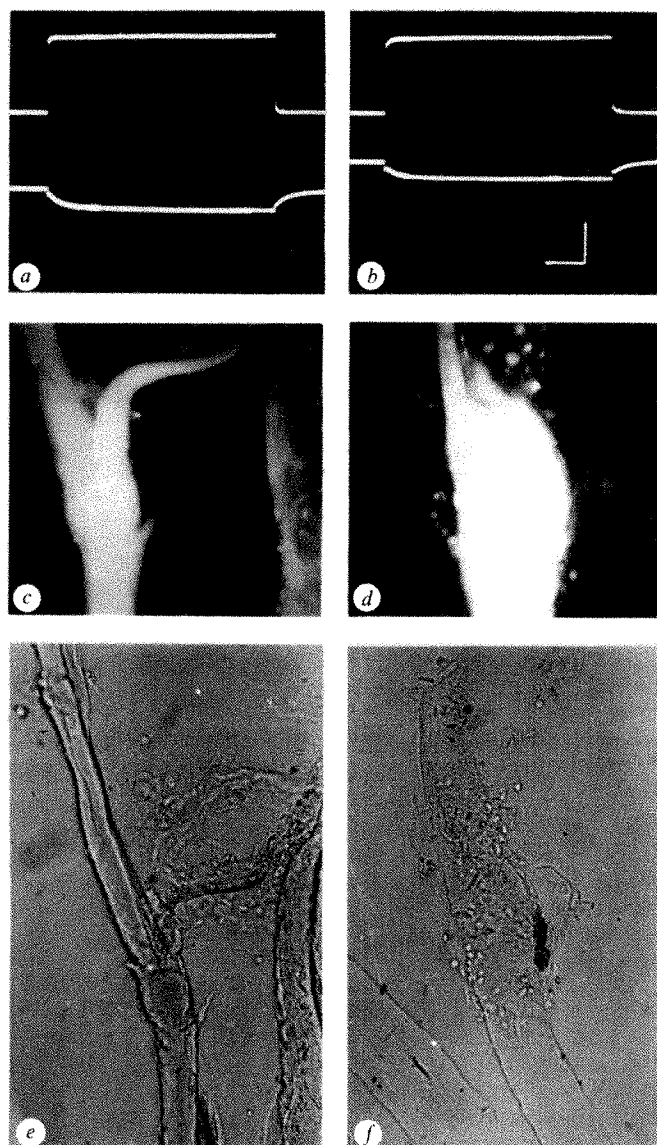
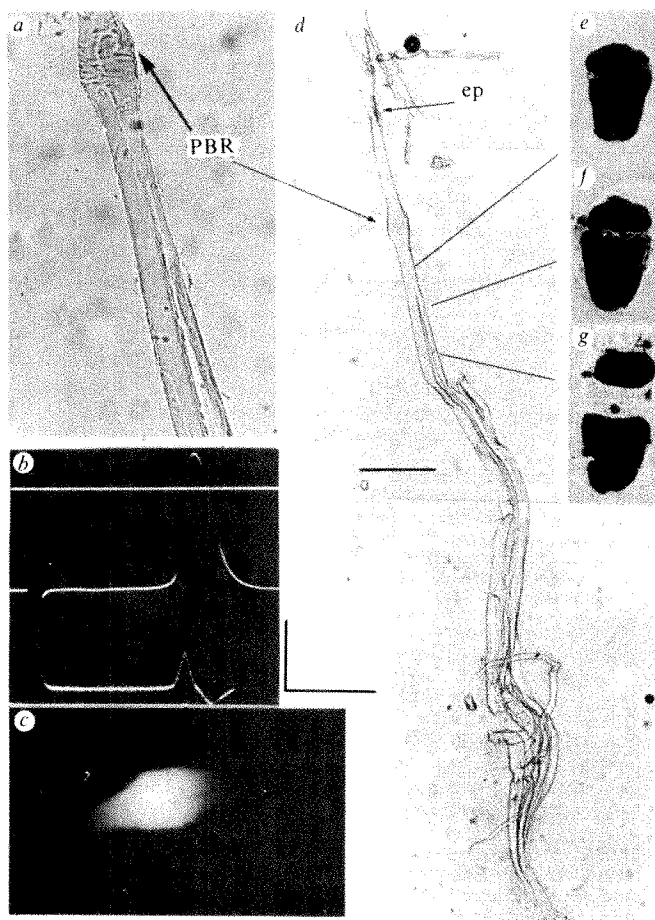


Fig. 1 Two typical experiments in which the input resistance of a muscle fibre was recorded using a standard two microelectrode technique. The current passing electrode was filled with a 4% solution of Procion brilliant red. The hyperpolarisation of the membrane (lower trace) in the two fibres following the passage of current (upper trace) is shown in *a* and *b*. The muscle fibres were then marked with dye by passing steady d.c. current for 10-20 s. The marked fibres were located using fluorescence microscopy (*c*, *d*) stained for cholinesterase and teased from the whole muscle (*e*, *f*). The first fibre (*a*, *c*, *e*) showed longitudinal splitting, and dye injection had taken place at the site of splitting. The second fibre (*b*, *d*, *f*) was 'short' and had probably undergone necrosis. M.e.p.s were recorded from this fibre (not shown). Calibrations; *a*, *b*, 5 ms horizontal; 20 nA, 20 mV vertical; *e*, *f*, 70 μ m.

Fig. 2 An indirect action potential with its first derivative (*b*) was recorded from a dystrophic muscle fibre. The fibre was subsequently marked with Procion brilliant red and located using fluorescence microscopy (*c*). After staining for cholinesterase and teasing (*a*, *d*) the endplate cholinesterase (ep) and the site of the dye injection (PBR) were located. This fibre displayed longitudinal splitting, confirmed by serial section of the embedded fibre (*e*, *f*, *g*). Calibrations; *b*, 50 mV, 500 Vs⁻¹ vertical; 5 ms horizontal; *d*, 80 μ m.



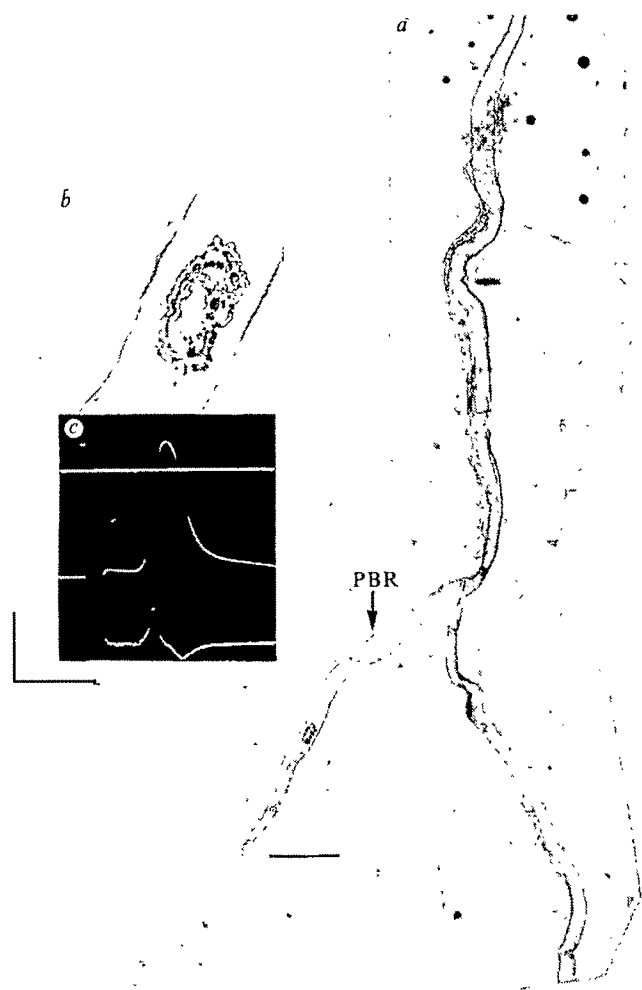


Fig. 3 An indirect action potential with its first derivative (*c*) was recorded from a dystrophic muscle fibre which was marked with dye (PBR in *a*). This fibre was very short, and it terminated before the normal tendon of insertion (*a*). Its cholinesterase distribution appeared normal (*b*). Calibration; *c*, 50 mV, 500 V s⁻¹ vertical; 3 ms horizontal; *a*, 80 μm.

brilliant red H3BN (ICI, Blackley) was introduced into the muscle fibre within 100 μm of the recording electrode. Dye was ejected by passing inward direct current (20–80 nA; 10–20 s) through the dye-filled microelectrode. A visible red spot and a localised swelling of the muscle fibre indicated successful staining. Muscles were immediately fixed in cold (4 °C) formal-calcium (10% formalin, 1% CaCl₂) for 4 h, teased into small bundles and stained for cholinesterase activity⁸. The marked fibres were located using a Vickers Photoplan fluorescence microscope. The exciting light was filtered through a combination of Schott UG3, Schott BG12 and Balzers TRITC-I filters; emitted light was collected using a combination of Schott GG4 and Wratten 2E barrier filters. The marked fibres, which fluoresced bright red, were teased from the bundles, and, after photography, were embedded in 1% agar-agar, post-fixed in phosphate-buffered 4% glutaraldehyde (1 h) followed by phosphate-buffered 1% osmium tetroxide (15 min), and were then routinely processed and embedded in Spurr resin for subsequent microtomy. Sections were cut at 1 μm and were stained with hot toluidine blue.

The experiments indicated that grossly abnormal fibres in the dystrophic muscle were sampled using the described techniques, and two of these fibres are shown in Fig. 1. In 12 fibres indirect action potentials were generated and the fibres were subsequently marked with dye and teased out of the muscle. Ten of the fibres showed abnormalities such as

Table 1 Mean quantum content of e.p.ps evoked at 3 Hz and 30 Hz nerve stimulation in uninjected fibres and fibres injected with Procion brilliant red

	Mean quantum content	
	3 Hz	30 Hz
Normal EDL (unmarked)	306.3 ± 22.5 (14)	184.2 ± 15.8 (14)
Normal EDL (marked)	346.2 ± 51.9 (5)	232.0 ± 35.4 (5)
Dystrophic EDL (unmarked)	381.9 ± 47.1 (22)	233.4 ± 24.4 (22)
Dystrophic EDL (marked)	379.0 ± 127.4 (12)	243.9 ± 40.9 (12)

Fibres were injected from microelectrodes filled with a 4% solution of dye. Figures are mean ± s.e.m. Mean values at corresponding frequencies are not significantly different ($P > 0.05$; Welch test).

longitudinal splitting^{9,10} (Fig. 2) or necrosis (Fig. 3). In further 12 muscle fibres, the quantum content of the evoked e.p.ps was shown to be normal (Table 1); yet nine of the fibres show overt morphological abnormalities (Fig. 4 is typical).

We have thus demonstrated normal transmission in 20 dystrophic muscle fibres, 19 of which were grossly abnormal exhibiting between them longitudinal fibre splitting, necrosis and, apparently, termination within the bulk of the muscle rather than at the normal myotendinous junction¹¹. We suggest, therefore, that the pathological involvement of muscle fibres in this disease cannot be either a direct or indirect consequence of a peripheral block of neuromuscular transmission.

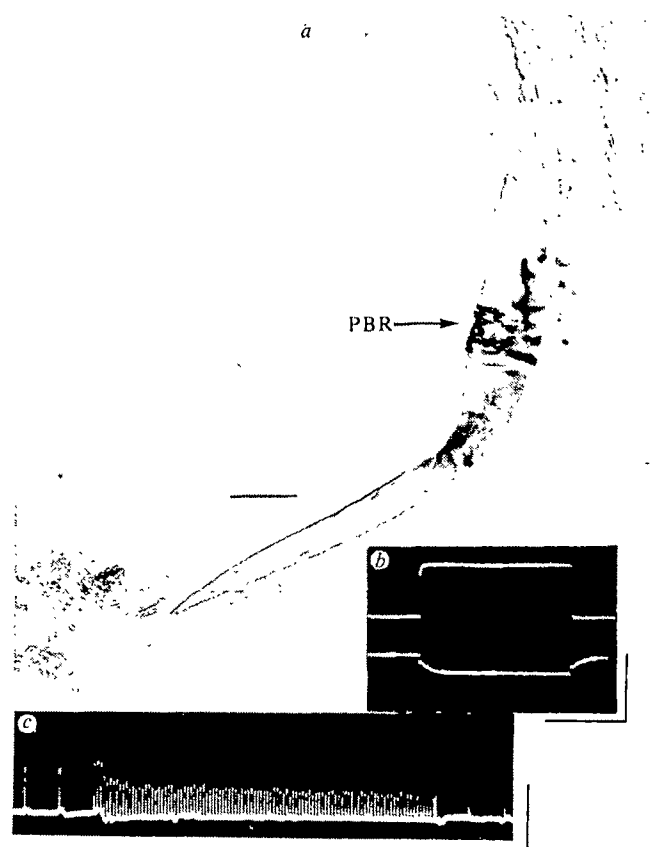


Fig. 4 Input resistance (*b*) and a train of endplate potentials (30 Hz, *c*) were recorded in the presence of *d*-tubocurarine from a dystrophic muscle fibre which was marked with dye (PBR in *a*). This fibre terminated well before the normal insertion of adjacent fibres. The cholinesterase staining was somewhat fragmented. The quantum content of the e.p.ps was normal (see Table 1). Calibration *a*, 50 μm; *b*, 60 nA, 60 mV vertical, 15 ms horizontal; *c*, 10 mV vertical, 1 s horizontal.

We acknowledge the support of the Muscular Dystrophy Group of Great Britain and the MRC. Richard Ribchester holds an MRC Studentship.

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Neuromuscular blockade increases motoneurone survival during normal cell death in the chick embryo

NATURALLY occurring neuronal cell death is a quantitatively significant event in many developing systems¹⁻⁵; however, the determinants responsible for some neurones surviving while an equal or even greater number degenerate are still unknown. Neurones which undergo spontaneous degeneration have axons in the appropriate peripheral area^{6,7}, and it is generally believed that those neurones which die do so because they are losers in a competition for a limited number of 'targets'^{11,8}, or a limited amount of 'trophic substance'^{9,10}. This study was undertaken in an attempt to better understand the parameters which define a 'target' and the factors involved in the survival and degeneration of motoneurones in the lateral motor column of the lumbar spinal cord of the chick. Normally by embryonic day 6 a peak of approximately 22,000-24,000 motoneurones is found in each lateral motor column whereas by day 9 there are 13,500 and by day 12, and continuing through hatching, each lateral motor column consists of 11,000-11,500 motoneurones (unpublished data, also see refs 3, 11). We report here that contrary to what might be expected¹², embryos treated during the period of normal cell death with botulinum toxin (BTX) or curare contain about 5,500 more motoneurones (a 50% increase) in each lateral motor column after the period of cell death is over. Treatment with the irreversible nicotinic receptor blocker α -cobratoxin (CT, from *Naja naja siamensis*) also produced an increase in motoneurone survival; however, no studies were carried out with this agent beyond day 10.

Drugs were administered either by intramuscular (i.m.) injection (0.05-0.25 μ l) into the right leg (ventral muscle mass) on day 6 using a 33 gauge Hamilton microsyringe fitted with a 40- μ m glass tip¹³ (CT, BTX, and sterilised BTX), or by dropping the solutions (0.1-0.25 ml) on to the vascularised chorioallantoic membrane (curare and CT) on days 6, 7, 8, and 9. Embryos were killed for cell counts either on day 10 or day 16. After treatment with pre- or postsynaptic blocking agents a single leg at day 16 was capable of maintaining 50% more motoneurones (Fig. 1, Table 1). This increase did not seem to be a transient effect, as there were comparable numbers of neurones present in treated embryos on days 10 and 16. Moreover, no increase in neurone survival was found in embryos that were injected with curare after the period of cell death was over (group curare II in Table 1). Also, there was no enhancement of neuronal survival when drug treatment was not effective for the entire period between days 6 and 10 (group CT II).

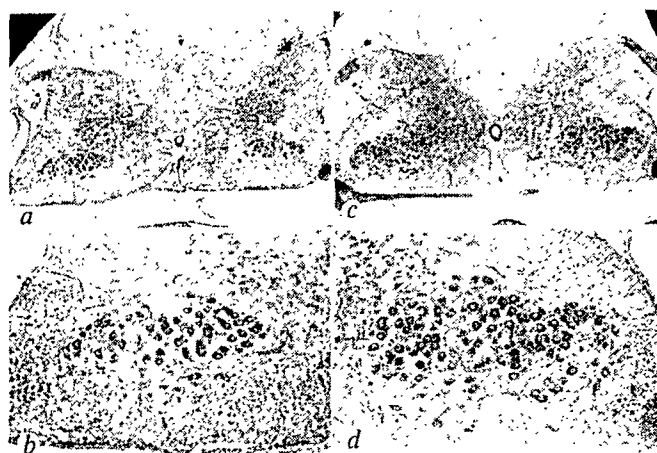


Fig. 1 Cross section through the spinal cord (fourth lumbar segment) of 16-d control (a, 20 \times ; b, 51 \times) and curare-treated (curare I; c, 20 \times ; d, 51 \times) embryos. The lateral motoneurones are the cluster of large dark staining cells in the ventro-lateral part of the cord. Spinal cords from BTX-treated embryos appear very similar to those from curarised embryos.

Curare, CT and BTX all decrease the amount of acetylcholine interacting with receptors, thereby reducing muscle contraction and motility. Motility levels are, therefore, a rough index of the drug's ability to interfere with normal neuromuscular function. All treatments which increased motoneurone survival produced either a partial or total inhibition of neuromuscular activity from day 6-10, and continued to decrease motility at least until day 16 (Table 1). Embryos in group CT II received a single injection of cobratoxin on day 6 which totally stopped all overt motility for the first half of the cell death period (day 6-7 $\frac{1}{2}$), whereas after day 8 motility levels were similar to controls. Interestingly, these embryos showed no increase in motoneurone numbers after killing on day 10. This suggests that either the critical period for increased cell survival occurs after day 8 or that once activity returns to near normal levels on day 8-8 $\frac{1}{2}$, 'redundant' synapses are lost, leading to a degeneration of the excess neurones.

The additional motoneurones present on day 16 seem to be uniformly distributed throughout the lateral motor column except for the most rostral segments (Fig. 2)—this distribution is very similar to the spatial distribution of 'hypothanasia' seen after transplanting additional limbs on to the body wall¹⁴. This may imply that virtually all muscles in the leg are capable of maintaining additional motoneurones. Other interpretations

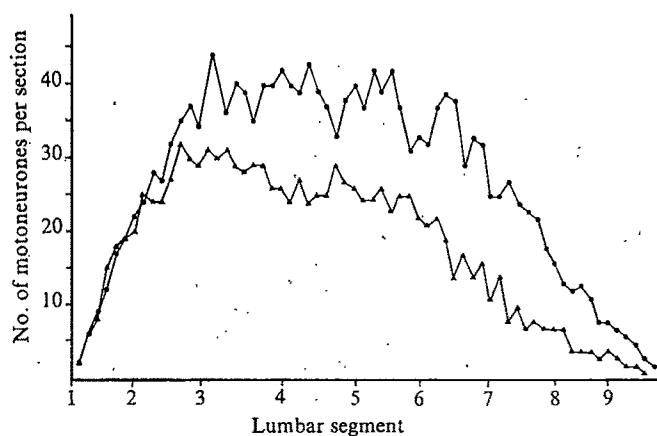


Fig. 2 Motoneurone counts in the right lateral motor column in lumbar segments 1-9 of 16-d control (▲) and curare-treated (●, curare I) embryos. Counts for every tenth section are represented. Note the increased cell number in segments 3-9 for curare-treated embryos. Motoneurone distribution after BTX treatment is basically the same as that after curare.

Table 1 Number of motoneurons and levels of embryonic activity after treatment with curare, botulinum toxin (BTX) and α -cobratoxin (CT)

Group* (age when killed)	Treatment	Motoneurons		% Change in motoneurone no.	Motility (moves min ⁻¹)				
		right LMC	left LMC		7	8	9	10	16
10 d control (5)		13,720 \pm 585	13,656 \pm 670	—	6.8 \pm 1.9	10.2 \pm 1.7	18.1 \pm 1.3	16.4 \pm 1.9	—
10 d sterilised									
BTX(6)	i.m., day 6	13,816 \pm 531	13,678 \pm 688	—	—	—	—	13.3 \pm 6.3	—
10 d curare(3)	2.5 mg on to CAM days 6, 7, 8, 9	17,067 \pm 127	—	+24	4.1 \pm 0.5	2.6 \pm 0.5	0.4 \pm 0.8	7.0 \pm 3.0	—
10 d BTX(4)	i.m. day 6; 2,000 LD ₅₀	—	17,080 \pm 704	+25	—	—	—	2.3 \pm 0.7	—
10 d CT I(3)	100 μ g, day 6; 85 μ g days 7, 8; 50 μ g day 9, on to CAM	16,657 \pm 710	—	+21	0	1.0 \pm 0.7	0	5.0 \pm 3.0	—
10 d CT II(8)	i.m. day 6; 6 μ g	13,398 \pm 836	13,387 \pm 816	-2 (both sides)	—	—	—	15.0 \pm 2.1	—
16 d control(4)	—	11,360 \pm 764	11,203 \pm 577	—	—	—	—	—	14.6 \pm 6.0
16 d BTX(5)	i.m. day 6; 1,200 LD ₅₀	—	16,402 \pm 438	+46	—	—	—	—	0
16 d Curare I(3)	2.5 mg on to CAM day 6, 7, 8, 9	17,597 \pm 1400	—	+55	1.8 \pm 1.3†	4.1 \pm 2.6	2.6 \pm 0.7	3.6 \pm 1.1‡	7.2 \pm 2.5
16 d Curare II(3)	4.0 mg on to CAM days 12, 13, 14, 15	11,537 \pm 923	—	+2	—	—	—	—	0

Data are expressed as the mean \pm s.d. Abbreviations: LMC, lateral motor column; CAM, chorioallantoic membrane; LD₅₀, 50% lethal dose for a 20g mouse. Weights of shank muscles (g) at day 16 were: control, 0.38 \pm 0.04; BTX, 0.27 \pm 0.02; curare, 0.26 \pm 0.05. Lumbar cords were run up for paraffin histology, serially sectioned at 12 μ m, stained in thionine and the motoneurons in either one or both LMCs were counted in every tenth section^{9,11}. For groups in which counts of only one LMC are presented, at least one animal in each group was counted on both sides to assure that no discrepancies existed between right and left LMCs following treatment. The injection procedure itself did not produce any noticeable motoneurone degeneration (see groups CT II, and sterilised BTX); however, as an added precaution, only the left LMC was counted in BTX embryos. Motility recordings for CT I and curare-treated embryos between day 6 and 10 were made just before the daily drug treatment (that is, 24 h after the last treatment). Previous observations¹⁹ have shown that 2.5 mg of curare stops all activity for much of the 24-h period, followed by a subsequent slow rise in motility. Therefore, the activity levels presented represent the maximum activity for the preceding 24-h period.

*Number in group given in parentheses.

†Sample size for motility recordings on days 7–15 was $n = 5$; for day 16, $n = 8$.

‡Embryos were also recorded between days 10 and 16 but for illustrative clarity the data are not shown here. The average rate of motility during this time was about 8 per min compared to 16–22 per min for controls.

are possible, however, such as a preferential alteration of either fast or slow muscle properties and/or an increased number of axons forming synapses outside their normal area of innervation.

Our data show that decreasing functional activity at the neuromuscular synapse is correlated with an increase in the survival of motoneurons normally destined to undergo natural cell death. As has been previously reported^{15,16} curare, BTX and CT produced muscular atrophy; therefore, the possibility that an increase in the size or number of muscle fibres is responsible for the maintenance of the additional neurones is unlikely. It seems most likely that muscle fibres, which in normal conditions would maintain only one neurone, are capable of accepting and maintaining additional neurones after treatment. One explanation for this may be that normally the first axon to contact the myotube alters the membrane-receptor characteristics such that those parts of the myotube membrane not adjacent to the nerve terminal are rendered refractory to other axons. By blocking this initial nerve-muscle interaction we may have altered this process so that the myotube becomes receptive to additional axons^{17–19}. Alternatively, it might be that once the axon contacts the myotube and initiates contractions, there is a period in normal development during which the muscle membrane properties (ACh receptor number or distribution?) are changing as a result of the increased activity. Before and during this critical period, more than one neurone could be maintained by each myotube; however, once the number of contractions reached a critical level, the membrane properties would be altered such that only one neurone could be 'supported', and additional contacts would be lost. By decreasing myotube contractions with curare, BTX or CT, the critical period would be maintained with a resulting increase in motoneurone survival. The normal number of motoneurons present on day 10 in embryos which had a motility deficit before but not after day 8 (group CT II) could be accounted for by either model. Preliminary studies

with embryos in which motility is depressed from day 6–10 by daily injections of cobratoxin indicate that once motility returns to control levels (day 12–13) the additional neurones that are present on day 10 are lost; a finding which is also consistent with both models. The critical difference between the two models is that in the latter, but not the former, contact of the myotube by more than one axon is postulated to be a normal, though transient, process. We are carrying out experiments that may distinguish between these two alternatives.

Although our results support the idea that it is a limiting of 'targets' rather than of 'trophic substance' that is the important factor in naturally occurring cell death, in the future it may be neither profitable nor possible to draw any useful distinction between these two. We therefore emphasise that the novelty of our results is that they strongly implicate physiological activity at the neuromuscular synapse in the control of cell survival. Our results also support the suggestion that neurones which undergo normal cell death may form connections before spontaneous degeneration^{1,3}.

This work was supported by the North Carolina Division of Mental Health Services, Research Section and NSF grant BMS 75-23327. We thank Carol Willard and I.-W. Chu-Wang for their assistance. Botulinum toxin was a gift from Dr E. J. Shantz.

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Received 6 September; accepted 21 November 1977.

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Gating properties of acetylcholine receptor in newly formed neuromuscular synapses

A FOREIGN motor nerve transplanted on to an adult innervated muscle will not form functional neuromuscular synapses¹, yet several experimental procedures, such as denervation or muscle crush, change the properties of the muscle membrane so that a foreign nerve can establish 'ectopic' synapses in a normally endplate-free region of the muscle²⁻⁴. The nerve thereby induces a localised area of high acetylcholine receptor (AChR) density in the extrajunctional membrane⁵. Denervated or previously crushed muscle fibres already have, before synapse formation, low density AChRs in the extrajunctional membrane^{6,7}. The extrajunctional AChRs are different in a number of respects from those in the junctional membrane⁸⁻¹¹. The question is, therefore, whether the AChRs of an ectopic synapse have properties similar to junctional or to extrajunctional AChRs.

The two types of AChR differ in their gating properties, since the mean open time of extrajunctional channels is about 3 to 5 times that of junctional channels¹²⁻¹⁴. We have therefore measured the mean open time and the mean conductance of ion channels of ectopic synapses in frog (*R. temporaria*) sartorius muscle by analysing acetylcholine (ACh)-induced membrane current noise^{11,15}. The results indicate that the gating properties of ion channels in ectopic synapses are similar to those of normal synapses.

The tibialis nerve was connected to the tibial endplate-free zone of sartorius muscle. At the same time the muscle was cut or crushed near the site of implantation^{3,4}. In the majority of experiments 2 to 3 months later a connective septum has formed at the site of muscle crush, isolating muscle fibres of about 3-5-mm length extending between the septum and the tibial myotendinous junction. Most of these fibres respond to electrical stimulation of the tibialis nerve with an action potential or subthreshold endplate potentials (e.p.ps), and in some fibres low frequency (< 1 min⁻¹) miniature endplate potentials (m.e.p.ps) can be recorded^{3,4}. Occasionally, doubly innervated fibres are encountered which can be stimulated both by the tibialis and the sartorius nerve.

Examination of ACh sensitivity of tibialis-innervated fibres by focal iontophoretic application of ACh along a fibre reveals a peak of sensitivity at the site of the ectopic synapse. When these muscles are exposed to ¹²⁵I- α -bungarotoxin (BuTX), in most fibres several patches of high grain density are seen in the autoradiogram. The shape of these patches is round or elongated and they range in size from 5 to 30 μ m. In some fibres where elongated patches are present, few or no grains at all are seen on the rest of the fibre (Fig. 1a). In other fibres the rest of the membrane is also strongly labelled. Similar patches of high grain density are also found in muscles in which the tibial nerve had failed to establish synapses, for example, as a result of nerve displacement after the operation (Fig. 1b). They probably correspond to 'hot spots'^{16,17} found also in chronically denervated frog cutaneous pectoris muscle (A. Michler, personal communication). At present, we have no clue as to which of the patches found in innervated muscles corresponds to an ectopic endplate and which to a 'hot spot'.

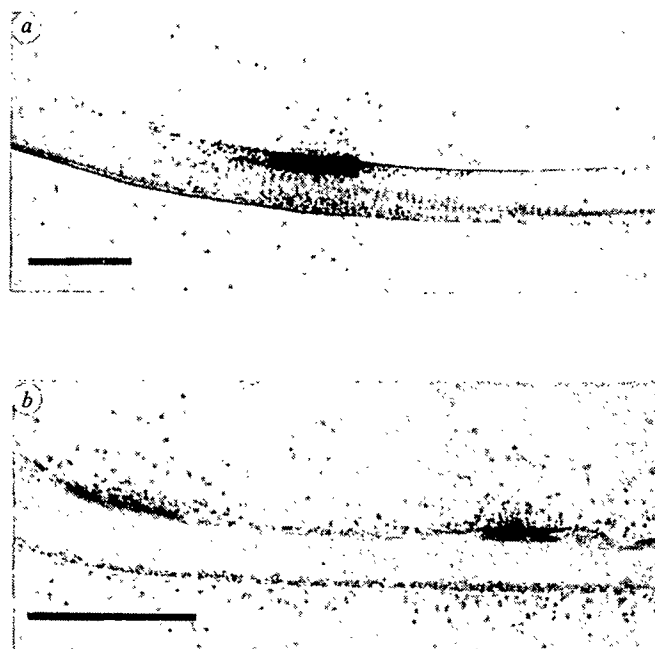


Fig. 1 Autoradiograms of muscle fibres isolated from the tibial portion of operated sartorius muscle. At the time of initial operation, the muscle was cut 5-10 mm away from its tibial insertion and the tibialis nerve was implanted into the remaining tibial portion. Several months later, muscles were exposed for 2 h to 1 μ g ml⁻¹ ¹²⁵I- α BuTX (specific activity: 4.7×10^5 Ci mol⁻¹) and subsequently fixed in glutaraldehyde. Single fibres were teased and dipped in Ilford L4 emulsion and exposed for 24 h. *a*, Elongated patch of high grain density in a fibre from a muscle which responded to stimulation of the tibialis nerve. Experiment 116 d after operation. *b*, Patches of high grain density ('hot spots') in a fibre from a muscle in which the tibialis nerve did not make contact as a result of mechanical displacement. The fibres were in effect chronically denervated for 89 d. Scale bar, 20 μ m.

A tentative hypothesis is that the elongated patches in fibres with otherwise low grain density (Fig. 1a) represent ectopic endplates.

To study their channel properties, superficial ectopic endplates were voltage clamped to a holding potential between -70 mV and -90 mV. Membrane currents of 15 to 130 nA mean amplitude were induced by releasing ACh from a micropipette located 30-50 μ m above the synapse. During ACh application, the clamp current fluctuates around its mean in a manner similar to that described for normal endplates. The autocorrelation function of ACh-induced current noise¹² shows a single decay constant τ_{noise} , the value of which varied between 1.36 ms and 1.85 ms in seven experiments carried out at 15-18 $^{\circ}$ C, the mean value being $\tau_{noise} = 1.5 \pm 0.3$ ms (\pm s.e.m.). The values of individual experiments were normalised to -80 mV membrane potential and 18 $^{\circ}$ C assuming¹⁵ a Q_{10} of 2.77 and an e -fold increase of τ per 80 mV hyperpolarisation. The mean value of τ_{noise} which is an estimate of the channel open time^{11,15} is thus very similar to that reported for normal synapses in *R. temporaria*¹². The result of one experiment is shown in Fig. 2a.

The size of the single channel current i obtained from the ratio of the variance σ^2 of the current fluctuations to the mean current I was more variable and ranged between 0.7 pA and 1.7 pA in these experiments. The reversal potential of nerve-evoked endplate currents (e.p.cs) in ectopic synapses was determined in three experiments and was found to be between -5 mV and 0 mV (Fig. 3). This is very similar to what has been reported as a reversal potential for e.p.cs evoked by nerve stimulation or iontophoretic ACh application at normal synapses^{18,19}. The size of the elementary conductance change has a mean value of $\gamma = 16 \pm 1.4$ pS (range 10-21 pS, seven experiments, reversal potential assumed at 0 mV) and is thus somewhat lower than the value obtained previously for normal synapses¹².

A possible reason for the lower estimate of γ in ectopic synapses as compared to normal synapses could have been that during ACh application an appreciable fraction of all available receptors is liganded, since AChE activity is low in these synapses. Therefore, the low concentration limit required for estimating i from the ratio σ^2/I might not hold and i would be underestimated²⁰. This was tested in one experiment by inducing mean membrane currents which varied in size over a fourfold range. In this experiment, the estimate of γ was independent of the magnitude of the mean current. Another possibility for the smaller estimate of γ might be that in most experiments a relatively high concentration of Mg^{2+} was present in the bathing solution.

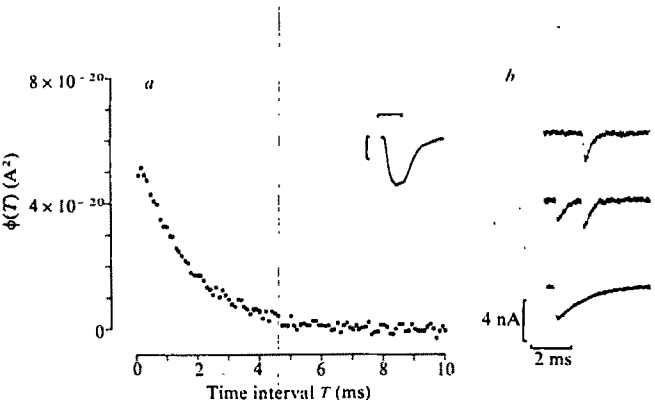


Fig. 2 Analysis of endplate currents at ectopic synapse. All experiments were carried out in frog Ringer solution of the following composition (in mM): NaCl, 114; KCl, 2.5; CaCl₂, 0.5–1; MgCl₂, 0–15; Na₂HPO₄, 2.15; NaH₂PO₄, 0.5; pH, 7.1. MgCl₂ was added in five out of seven experiments to abolish nerve-evoked muscle contraction. Superficial ectopic synapses were located by the rise time of e.p.ps 97 and by applying hypertonic sucrose from a blunt micropipette along the fibre. A brisk increase in m.e.p.p. frequency indicated the site of innervation. *a*, The graph shows the autocorrelation function of ACh-induced voltage clamp current fluctuations. Data points represent value of autocorrelation function $\Phi(T)$ for different time intervals T . Time course of endplate current during iontophoretic ACh application is shown in the inset (calibration marks indicate 20 nA and 25 s). A current sample of 6.4 s duration, taken during the peak of the response, was used for calculation of autocorrelation function. Mean amplitude: $I = 43$ nA. The record was filtered with a band pass (0.5 Hz–2.5 kHz). Sampling interval 100 μ s. Control data obtained from a current sample in the absence of ACh was subtracted. Continuous line is an exponential function fitted by eye to the data points. It yields a value of $\tau = 1.6$ ms for the time constant and an extrapolated ordinal intercept of $\sigma^2 = 6.1 \times 10^{-20} A^2$ for the variance of ACh-induced current fluctuations. Holding potential: -80 mV. Temperature $18^\circ C$. Experiment performed 104 d after implantation of the tibialis nerve. *b*, Upper two traces: Examples of m.e.p.cs recorded from the same fibre as in (*a*), $\tau_{m.e.p.c.} = 3.4$ ms. Bottom trace: m.e.p.c. from another fibre with extremely long decay time $\tau_{m.e.p.c.} = 12$ ms at -80 mV membrane potential and $18^\circ C$. Time constant of current fluctuations in this fibre was $\tau_{noise} = 1.8$ ms.

Whereas the elementary conductance events in normal and ectopic synapses are similar, the time course of miniature endplate currents (m.e.p.cs) is different in the two types of synapses. In a normal synapse the m.e.p.c. decay time constant $\tau_{m.e.p.c.}$ is similar or identical to the mean open time of ion channels as derived from noise analysis¹⁵. In all ectopic synapses studied, $\tau_{m.e.p.c.}$ was longer by a factor of 2 to 6 than τ_{noise} and ranged from 3.3 ms to 12.6 ms (see Fig. 2*b*, Table 1). In contrast to the relative uniformity of τ_{noise} in different fibres, the value of $\tau_{m.e.p.c.}$ changed from fibre to fibre. This is similar to what is observed at normal endplates after inhibition of AChE, suggesting that the AChE activity in ectopic synapses is low compared to that in a normal synapse. In most ectopic synapses we failed to detect AChE activity histochemically by the Karnovsky method²¹, even after several hours of incubation and despite intense staining of myotendinous junctions which were only a few millimetres away. We also found in three experiments that adding 3–6 μ M neostigmine

Fig. 3 Determination of reversal potential of nerve-evoked e.p.c. in a newly formed synapse. Membrane potential was clamped to the value indicated beside each trace and 20–50 responses were averaged at each potential level. Arrow indicates time of tibialis nerve stimulation. E.p.c. reverses sign between -5 mV and 0 mV membrane potential. Experiment was carried out 89 d after operation.

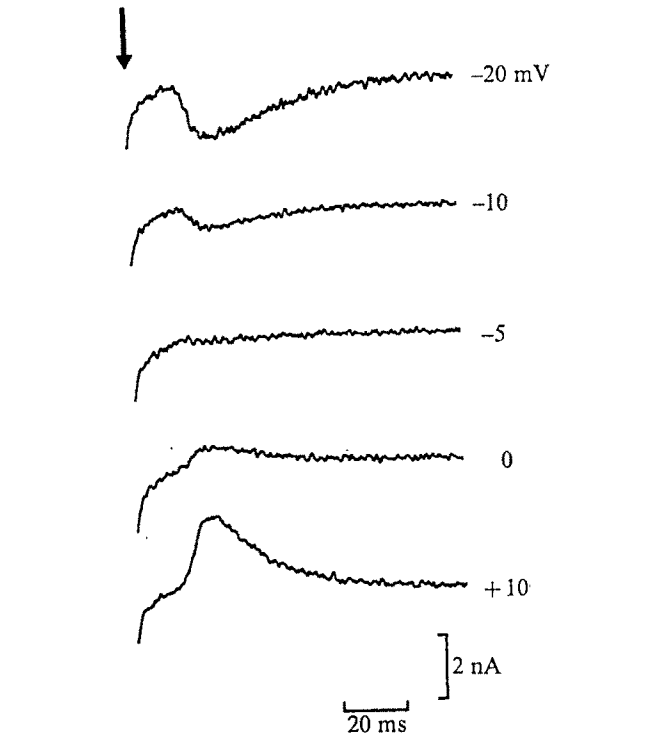


Table 1 Comparison of mean channel open time and mean m.e.p.c. decay time constants obtained from the same ectopic synapse					
Experiment	τ_{noise} (ms)	$\tau_{m.e.p.c.} \pm s.e.m.$ (ms)	Membrane potential (mV)	Temperature ($^\circ C$)	Time after operation (d)
1	1.58	3.76 (0.09)	-80	17	90
2	1.82	5.14 (0.21)	-70	15	91
3	1.85	12.23 (0.35)	-80	18	93
*4	1.61	3.36 (0.29)	-80	18	115
*5	1.52	6.96 (0.35)	-80	18	113
6	1.71	—	-70	16	81
7	1.45	4.22 (0.15)	-90	16	97

$\tau_{m.e.p.c.}$ represents mean values obtained from 9 to 25 individual m.e.p.cs. Time after operation refers to the time between implantation of the tibialis nerve and the time the experiment was carried out.
*Experiments done in frog Ringer solution with no Mg^{2+} ions present. In the remainder Mg^{2+} concentration varied between 5 and 15 mM.

to the bath prolonged $\tau_{m, e.p.c.}$ by a factor of less than 1.25 as compared to a factor of 2 reported for normal neuromuscular synapses^{22,23}.

We have shown that ion channels associated with AChRs of newly formed ectopic synapses on adult muscle are similar in their gating properties to those found in normal endplates. They are different from those of extrajunctional AChRs found in denervated fibres not contacted by a nerve. The question arises as to whether, during synapse formation in adult fibres, extrajunctional AChRs are accumulated under the influence of the motor nerve, as has been shown recently for embryonic amphibian muscle²⁴ and are changed in their properties to junctional AChRs, or whether the motor nerve induces synthesis of 'junctional' receptors at its site of contact. Experiments carried out at early stages of synapse formation may help to answer this question.

We thank Professor J. Schmidt for ¹²⁵I- α -BuTX and Ms A. Michler for help with autoradiography. H. R. Brenner was supported by the Schweizerische Nationalfonds.

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Received 28 October; accepted 1 November 1977.

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Direct or proximate contact between cells and metabolic activation systems is required for mutagenesis

THE adverse biological effects of most carcinogens and/or mutagens are dependent on the formation of electrophilic intermediates which react with nucleophilic groups in cellular macromolecules. Inclusion of a metabolic activation system in various assays enables the detection of mutagenic activity for carcinogens and/or mutagens which require such metabolic activation. In mutagenesis assays, using mammalian cells, two metabolic activation systems have been used: cell-mediated mutagenesis (CMM), in which cells are co-cultured with metabolically competent, but lethally irradiated, cells (feeder layer)^{1,2}, and microsome-mediated mutagenesis (MMM), in which cells are treated with chemicals in the presence of a microsomal fraction from rodent livers and an NADPH-generating system^{3–5}. The present study was carried out in order to investigate whether direct or proximate contact between target cells and such mediators of metabolism is necessary for the induction of mutagenesis. The results suggest that such contact is essential in both cell- and microsome-mediated mutagenesis of mammalian cells and, possibly, also in bacterial systems.

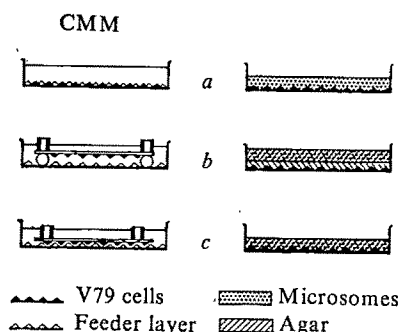


Fig. 1 Schematic representation of the experiments using CMM or MMM systems, which were designed either to allow or to prevent contact between the V79 cells and metabolic activation systems. Experimental procedures for CMM were adopted from Huberman and Sachs¹. Following irradiation at 4,000 R, secondary cultures of BDV1 rat embryo cells were plated at 2.5×10^6 cells per 60-mm dish (feeder layer) and, 5 h later, 3.0×10^6 V79 cells were plated and cultured overnight in Eagle minimum essential medium plus 10% foetal calf serum (FCS). The next day, cultures were treated with 7,8-diol BP (obtained from Dr P. L. Grover, Chester Beatty Research Institute), dissolved in DMSO, at concentrations of 1 and 2 nmol ml⁻¹ (8 ml per dish) for 48 h, followed by plating for determination of induced cytotoxicity and mutagenicity. Experimental procedures for MMM have been described elsewhere⁵; in brief, the V79 cells (overnight culture after plating 1.5×10^6 cells per 60-mm dish) were incubated for 2 h in 2.5 ml of the reaction mixture, either in liquid suspension or suspended in 0.5% agar. The reaction mixture consisted of 0.75 ml post-mitochondrial fraction from phenobarbitone-pretreated BDV1 rat livers (1 mg ml⁻¹ in drinking water for 7 d), 0.75 ml modified Sørensen phosphate buffer (0.055 M, pH 7.4, containing 0.9% NaCl and 1.6 mg ml⁻¹ MgCl₂·6H₂O) and 1.0 ml PBS containing 12.5 μ mol glucose-6-phosphate and 2 μ mol NADP⁺. The cells were then washed twice and incubated for 2–3 h in fresh culture medium, followed by plating for determination of induced cytotoxicity and mutagenicity. Cytotoxicity was determined by plating 100 cells per 60-mm dish (four dishes at each point) and culturing for 7 d. For mutagenesis, 2×10^4 and 10^5 cells per 60-mm dish were plated for *aza* and *oua* respectively (eight dishes at each point). After an expression period of 2 d, selection drugs were added to give final concentrations of 20 μ g ml⁻¹ 8-azaguanine or 1 mM ouabain. The media containing drugs were changed once, 5–7 d later; for the 8-azaguanine medium, FCS was replaced by dialysed FCS and non-essential amino acids were added at 0.1 mM. The cultures were fixed and stained with Giemsa 12 d (*aza*) or 14 d (*oua*) after plating. Mutation frequency was calculated per 10^5 survivors, taking into account the number of cells plated and the plating efficiency. The stability of *aza* and *oua* was demonstrated using 10 and 11 isolated colonies, respectively, which were cultured for more than one month in the absence of the drug used for selection⁶. *a*, Conventional CMM and MMM systems, as described above, in which the V79 cells are allowed to remain directly in contact with the cells or microsomes carrying metabolic activating enzymes. *b*, Is designed to separate the V79 cells from the metabolic activation system. In the CMM system, a glass cover slip (24 \times 40 mm) on which 1.4×10^5 V79 cells were plated and cultured overnight, was supported above the feeder layer at a distance of approximately 1 mm. In the MMM system, 2 ml of 0.5% agar in PBS was poured on the V79 monolayer (approximately 1 mm in thickness) and, after hardening, the reaction mixture suspended in 0.5% agar was added. *c*, In the CMM system, the V79 monolayer was grown on a cover slip and placed directly on the feeder layer. In the MMM system, the reaction mixture was suspended in 0.5% agar and poured directly on the V79 monolayer.

Mutations in two genetic loci, comprising 8-azaguanine resistance (*aza*) and ouabain resistance (*oua*), were studied using V79 Chinese hamster cells. Mutations were induced either by *trans*-7,8-dihydroxy-7,8-dihydrobenzo[*a*]pyrene (7,8-diol BP) at concentrations of 1 and 2 nmol ml⁻¹ in the CMM system, or by *N*-nitrosodimethylamine (DMN) at concentrations of 10 and 30 μ mol ml⁻¹ in the MMM system. Both these chemicals require metabolic activation^{3,5}. As shown in Fig. 1, the following experiments were designed either to allow, or to prevent, contact between the V79 cells and the feeder layer, or the microsomes carrying the enzymes responsible for metabolic activation: *a*, conventional mutagenesis assays

were used. In the CMM system the V79 cells were grown directly on the feeder layer of rat embryo cells and, in the MMM system, the cells were incubated in the presence of a reaction mixture containing a post-mitochondrial liver fraction from phenobarbitone-pretreated rats and co-factors; *b*, to separate the V79 cells from the feeder layer in the CMM system, a glass cover slip, on which a monolayer of the V79 cells had been grown, was placed above the feeder layer at a distance of approximately 1 mm. In order to maintain a constant distance between the two cell layers, the cover slip was supported by two strips of silicone rubber and pressed down with two metal cylinders used for colony isolation. In the MMM system, the V79 cells were separated by an agar layer (0.5% in phosphate-buffered saline, PBS) at a distance of approximately 1 mm from the reaction mixture, suspended in agar; *c*, this was designed as a control of the experimental conditions described in *b*. In the CMM system, the V79 monolayer, on a cover slip, was placed face down, directly on the feeder layer and was pressed down with two metal cylinders to ensure maximum contact between the two cell layers. In the MMM system, the reaction mixture suspended in agar was poured directly on to the V79 monolayer.

Figure 2 shows the mutagenicity of 7,8-diol BP in CMM; it produced *aza* and *oua* in a dose-related fashion when the V79 cells were cultured on the feeder layer in the conventional CMM assay (*a* in Fig. 1). No mutations were induced, however, either when the V79 cells were separated at a distance of approximately 1 mm from the feeder layer (*b* in Fig. 2), or when the V79 monolayer grown on the glass cover slip was placed directly on to the feeder layer (*c* in Fig. 1). In the latter, growth of the V79 cells was considerably reduced, presumably due to lack of circulation of nutrients under the cover slip; doubling time was more than 60 h compared with 15–16 h for the other systems (*a* and *b*). The results indicate that 7,8-diol BP, one of the metabolites of BP, is further metabolised in the CMM assay to an ultimate form, possibly 7,8-dihydroxy-9,10-epoxy-7,8,9,10-tetrahydrobenzo[*a*]pyrene (7,8-diol-9,10-oxide BP)⁶⁻⁸, which could be transferred to the target cells through direct or proximate contact with the feeder layer.

Similar results were obtained in the MMM assay (Fig. 3). Treatment of the cells with DMN in the presence of post-mitochondrial liver fraction and cofactors, either in liquid (*a* in Fig. 1), or in agar (*c* in Fig. 1), induced dose-related cytotoxicity and mutagenicity. However, neither cytotoxicity nor mutagenicity were induced when the cells were separated by the agar layer approximately 1 mm thick (*b* in Fig. 1). This lack of induction could be explained either by the requirement of direct contact or close proximity between the target V79 cells and microsomes carrying metabolising enzymes, or by the decomposition or trapping of methylating intermediates formed from DMN in the separating agar layer. The former possibility is further suggested by a conventional MMM assay in which the V79 monolayer was treated with DMN at 30 $\mu\text{mol ml}^{-1}$ in different volumes of the reaction mixture in

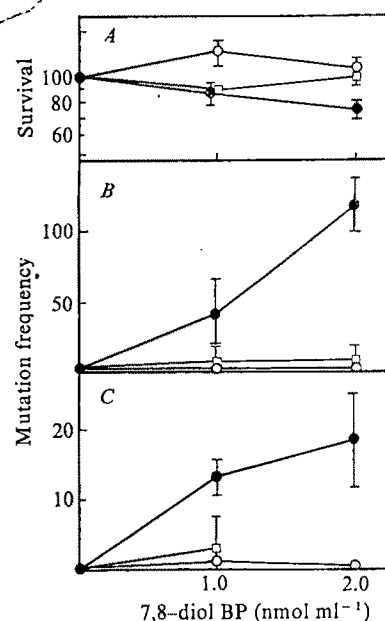


Fig. 2 Cytotoxicity (A) and mutagenicity (B, *aza*; C, *oua*) induced by 7,8-diol BP at concentrations of 1 and 2 nmol ml^{-1} in the CMM assay. ●, The conventional CMM assay (*a* in Fig. 1) in which the V79 cell grew directly on the feeder layer. ○, *b* in Fig. 1, in which the V79 cells were separated from the feeder layer. □, *c* in Fig. 1, in which the V79 monolayer was placed on the feeder layer. Cytotoxicity was expressed as percentages of plating efficiency of the solvent (DMSO) control. Mutation frequencies were expressed as the number of drug resistant clones per 10^5 survivors. *oua* in Fig. 1c at 2 nmol ml^{-1} was not examined. Bars indicate s.e.; when not indicated, s.e. are within the circles.

liquid (0.5, 1.5 and conventional 2.5 ml, which correspond approximately to 0.25, 0.75 and 1.25 mm in depth, respectively). The mutation frequencies obtained (mean number of resistant clones per 10^5 survivors, \pm s.e.m.) were 173 ± 18 , 212 ± 26 and 171 ± 19 for *aza* and 41 ± 4 , 60 ± 5 and 50 ± 4 for *oua*, respectively indicating that induction of mutations may take place independently of the total volume, in the thin layer of the reaction mixture.

In the experiment shown in Table 1, V79 cells were treated with a directly acting mutagen, *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine (MNNG), while separated from the MNNG-containing agar medium by an agar layer of varying thickness (approximately 0.5, 1.0 and 1.5 mm). No decreases in the induction of cytotoxicity and mutagenicity were observed when the cells were separated from the MNNG medium. There was also no difference in MNNG-induced mutation frequency between liquid and agar medium. These results indicate that agar did not trap or interfere with the diffusion of MNNG,

Table 1 MNNG-induced cytotoxicity and mutagenicity when the cells were separated from MNNG-containing agar medium by agar layers of varying thickness

Treatment		Agar layer for the separation		P.E. (%)	Mutation frequency	
		volume (ml)	thickness (mm)		<i>aza</i>	<i>oua</i>
DMSO	liquid	0	0	93.5 ± 4.8	4.0 ± 1.9	0
MNNG	liquid	0	0	66.0 ± 2.9	46.6 ± 3.6	4.7 ± 1.0
MNNG	agar	0	0	68.3 ± 7.7	46.6 ± 5.8	4.6 ± 1.2
MNNG	agar	1	0.5	58.3 ± 4.4	47.3 ± 6.5	5.2 ± 1.3
MNNG	agar	2	1.0	65.8 ± 4.0	50.4 ± 5.4	4.8 ± 1.4
MNNG	agar	3	1.5	51.8 ± 2.0	66.7 ± 9.1	11.1 ± 1.6

V79 cells were plated at 1.5×10^6 cells per 60-mm dish, cultured overnight and then treated with MNNG at 4 nmol ml^{-1} (4 ml per dish), either in liquid medium, or in 0.5% agar medium, for 2 h. The cells were separated from the MNNG-containing medium by agar layers (0.5% in phosphate-buffered saline (PBS), approximately 0.5, 1.0 and 1.5 mm in thickness, corresponding to 1.0, 2.0 and 3.0 ml of agar suspension. The cultures were then washed three times and cultured in a fresh culture medium for 2–3 h, followed by plating for determination of induced cytotoxicity and mutagenicity (see the legend for Fig. 1). This replating procedure produced a lower mutation frequency, approximately 20% for *aza* and 8% for *oua*, as compared to the conventional *in situ* treatment.

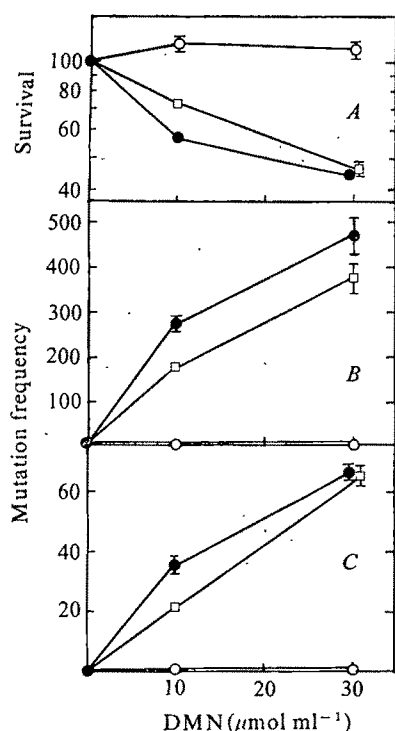


Fig. 3 Cytotoxicity (A) and mutagenicity (B, aza; C, oua) induced by DMN at concentrations of 10 and 30 $\mu\text{mol ml}^{-1}$ in the MMM assay. ●, The conventional MMM assay (a in Fig. 1) in which the V79 monolayer was incubated in the presence of the reaction mixture in liquid suspension. ○, b In Fig. 1, in which the V79 cells were separated by an agar layer from the reaction mixture suspended in agar. □, c In Fig. 1, in which the reaction mixture suspended in agar was poured directly on to the V79 monolayer. Cytotoxicity and mutagenicity are expressed as described in the legend to Fig. 2.

an alkylating agent that is relatively stable (half life is 90 min^{10,11}).

These results suggest that direct or proximate contact between the target cells and membranes of the cells or microsomal particles, carrying carcinogen-metabolising enzymes is essential in cell- and microsome-mediated mutagenesis of mammalian cells. This requirement may also hold true for mutagenesis assays using bacteria and microsomes. Glatt and Oesch¹², using electron microscopy, have demonstrated tight binding of microsomal particles to *Salmonella typhimurium* TA98, suggesting the importance of such a direct contact in bacterial systems.

Two mechanisms are plausible in explaining why direct or proximate contact is required. One is the chemical instability of the ultimate reactive metabolites formed, which, in many cases, are short-lived intermediates. For example, the half lives of synthetic 7,8-diol-9,10-oxide BP, an ultimate form of 7,8-diol BP, is 0.5 min for the *syn* isomer and 2.5 min for the *anti* isomer in aqueous media⁹; the half life of methylating intermediate(s) formed from DMN by liver microsomal monooxygenases was measured to be of the order of seconds (A. Barbin & H. Bartsch, personal communication). Such unstable compounds may thus decompose in aqueous media before reaching the DNA of the target cells, unless the distance of migration is reduced to a minimum. Second, when ultimate metabolites with lipophilic properties are formed, they could reach the target cells by transfer through the lipid matrix of membranes. This would require direct contact between the target cells and the metabolic activation systems. Such lipophilic environments may eventually contribute to a prolonged half-life of reactive compounds and may prevent contact with inactivating enzymes, such as glutathione S-transferases present in the cytosol¹².

We thank Drs H. Bartsch and R. Montesano and Messrs C. Malaveille and J. D. Wilbourn for their useful comments.

This work was supported in part by the US NCI (contract 1CP-55630).

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Received 23 August; accepted 21 November 1977.

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Ascorbate-enhanced cytotoxicity of misonidazole

MISONIDAZOLE, an electron-affinic, nitroheterocyclic compound (1-(2-nitro-1-imidazole)-3 methoxy-2-propanol, Ro-07-0582) is undergoing preliminary clinical trials, which are exploring its capacity to sensitise hypoxic tumour cells to ionising radiation damage¹⁻⁴. The drug also possesses a substantial cytotoxic effect, independent of radiation, which is selectively expressed in hypoxic cells^{5,6}. Both of these properties may contribute to the potential value of this agent as an adjunct to radiation therapy of malignant disease in those situations where radioresistant hypoxic cells represent the limiting factor in achieving local control. It is also possible that the cytotoxic property could be a useful adjunct to chemotherapy as it should be effective against noncycling hypoxic tumour cells. Misonidazole, however, may be cytotoxic to the normal hypoxic tissues in the human body (nerve, skin, cartilage, and so on) and this is a major concern in the clinical application of the drug^{1,4}. Furthermore, misonidazole is metabolised in mammalian cells and at least two products have been isolated⁷, toxic to both aerobic and hypoxic mammalian cells. Thus, if they are able to diffuse freely, the metabolites may also be a threat to aerobic tissues⁸. This cytotoxicity of misonidazole is temperature dependent^{9,10}, and varies with cell line^{11,12}, and cysteamine seems to protect against it¹³. It leads to strand breaks in cellular DNA and those cells which fail to survive also fail to repair these strand breaks¹². In this work we show the effect of ascorbic acid (vitamin C) on the cytotoxicity of misonidazole.

Chinese hamster ovary cells (CHO) were grown in suspension culture according to standard procedures⁵. Growth medium containing the drugs to be tested was de-oxygenated in a stirred glass vessel by flowing purified nitrogen over the solution for 10-15 min before the addition of hypoxic cells (2×10^5 cells ml^{-1}). The flow of nitrogen was maintained throughout the experiment. At the prescribed times, samples were withdrawn from the suspension, diluted, washed and plated in plastic culture dishes for the assay of colony forming ability⁵.

The effect of ascorbate on the hypoxic cytotoxicity of misonidazole is shown in Fig. 1. The toxicity of misonidazole alone in CHO cells is concentration dependent; it shows minimal toxicity to these cells at 5 mM while at 15 mM a 3-h exposure inactivates 95% of the cells. When 5 mM ascorbate is added to either 5 mM or 15 mM misonidazole there is a substantial increase in cell inactivation, although ascorbate alone is nontoxic to hypoxic cells. The

combined treatment of 5 mM ascorbate and 5 mM misonidazole is considerably more toxic than 15 mM misonidazole alone.

In contrast to its effect on hypoxic cells, ascorbic acid is very toxic to aerobic cells^{14,15}. This effect in CHO cells is shown in Fig. 2. Ascorbate oxidation involves one-electron transfer reactions which apparently result in the formation of hydrogen peroxide (H_2O_2) (ref. 16). The addition of catalase, an enzyme which blocks the accumulation of H_2O_2 , completely eliminates the aerobic toxicity of ascorbate to CHO cells. The addition of a nonspecific protein, bovine serum albumin, did not reduce the toxicity (data not shown).

Since ascorbate is an effective reducing agent, the increased toxicity of the combined misonidazole-ascorbate treatment of hypoxic cells could be due to accelerated formation of toxic, reduced metabolites of misonidazole. Alternatively, it might be argued that under hypoxia, misonidazole acts as an oxidant of ascorbate, leading to the formation of H_2O_2 and its resultant toxicity. But, catalase does not reduce the hypoxic toxicity of the misonidazole-ascorbate combination (Fig. 2). Furthermore, when gulonolactone, a nonreducing analogue of ascorbate, was substituted for ascorbate, there was no enhancement of misonidazole toxicity. It seems likely, therefore, that ascorbate promotes the reduction of misonidazole, leading to a greater accumulation of toxic derivatives. Another possible explanation is that, although ascorbate itself is not toxic to hypoxic cells it could somehow be predisposing cells to misonidazole damage. This possibility has not yet been examined.

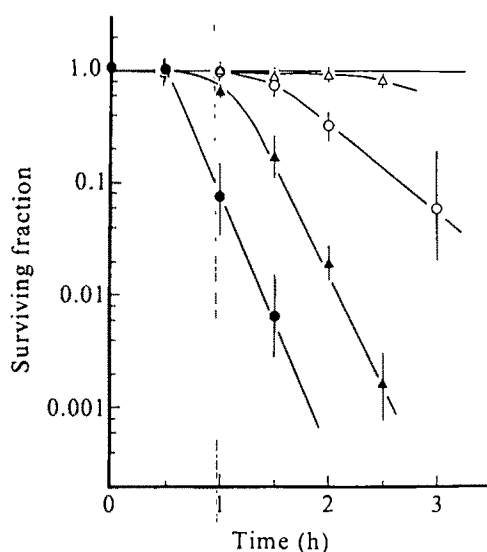


Fig. 1 Exponentially growing CHO cells were collected from suspension culture by centrifugation and resuspended in growth medium (MEM Alpha, supplemented with 10% foetal calf serum) at 6×10^6 cells ml^{-1} . After maintaining them at this concentration for 10–15 min at room temperature they were added to pre-gassed glass vessels containing growth medium and a prescribed amount of misonidazole and/or ascorbic acid such that the final concentration of cells was 2×10^6 cells ml^{-1} . Drug-medium solutions were freshly prepared before each experiment. The pH was adjusted to approximately 7.3 and solutions were sterile filtered. Purified N_2 , containing less than 5 p.p.m. O_2 , was flowed for 15 min over the stirred solutions at 37 °C before the addition of 1 ml of cells. The flow of N_2 continued throughout the exposure time. At the indicated times 1 ml samples were withdrawn and immediately diluted 10 times in aerobic growth medium at 0 °C. The cells were then washed once and plated for colony formation. Δ , 5 mM misonidazole; \circ , 15 mM misonidazole; \blacktriangle , 5 mM misonidazole plus 5 mM ascorbate; \bullet , 15 mM misonidazole plus 5 mM ascorbate. The error bars represent the s.e.m. values derived from pooled experiments.

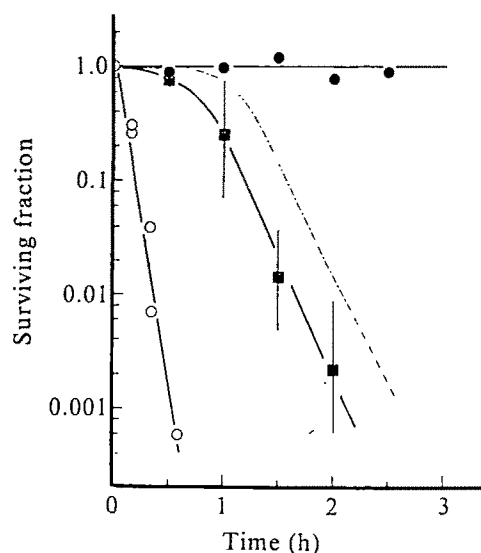


Fig. 2 The same procedure was used as described in Fig. 1, except that O_2 was flowed where indicated instead of purified N_2 . \circ , 5 mM ascorbate, O_2 ; \bullet , 5 mM ascorbate plus 0.3 mg ml^{-1} catalase, O_2 ; \blacksquare , 5 mM misonidazole plus 5 mM ascorbate and 0.3 mg ml^{-1} of catalase, N_2 . The dotted line represents the 5 mM misonidazole plus 5 mM ascorbate response in N_2 from Fig. 1. Under N_2 , the presence of catalase (or bovine serum albumin) increased slightly the toxic effects of the misonidazole-ascorbate combination in each experiment.

Several other aspects of this effect of ascorbate have been studied. In order to determine whether the supposed reaction between misonidazole and ascorbate required the presence of cells, ascorbate and misonidazole were added to the growth medium for up to 2 h before addition of cells. This did not change the combined response of Fig. 1, suggesting that the effect is a cell-mediated reaction, and does not occur spontaneously. The enhanced toxicity was found to be strongly temperature dependent. It is more rapid at 42.5 °C and almost absent at 0 °C. Variation of ascorbate concentration at a fixed misonidazole concentration, changes the duration of the initial shoulder seen in Fig. 1, but has little effect on the slope of the exponential portion of the curve. A similar potentiation of misonidazole toxicity was observed in the presence of reduced glutathione, the cofactor in the enzymatic oxidation of ascorbate; however, glutathione was less effective on a molar basis than ascorbate.

The effect of ascorbate and other reducing species (such as glutathione, cysteine, cysteamine) on the hypoxic cytotoxicity of misonidazole clearly requires further examination in order to assess and regulate any possible role in clinical applications. Misonidazole doses used in current clinical protocols result in plasma and tissue concentrations approaching 1 mM¹. The concentration of ascorbate (and perhaps other reducing agents) in the human body can also reach this range¹⁷, where the above *in vitro* studies suggest the occurrence of significant synergistic interactions. Whether the net effect of such interactions might be primarily positive (a contribution to the chemotherapeutic properties of misonidazole) or negative (an enhancement of the drug's cytotoxicity) cannot be predicted. The possibility, however, that such interactions may contribute to the problems of peripheral neuropathy, which have been described in reports of clinical trials of the drug⁴, needs to be explored. *In vivo* studies in experimental animals are a logical next step.

We thank Mrs Isabel Harrison and Mr Spencer Kong for technical assistance. The misonidazole was supplied by Dr C. E. Smithen of Roche Products Ltd. This work was

supported by the BC Cancer Foundation and the National Cancer Institute of Canada. P.D.J. is the holder of a NRC postgraduate scholarship.

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Received 24 October; accepted 17 November 1977.

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Novel treatment for joint inflammation

THE use of liposomes in the entrapment and administration of several therapeutic agents has been described for the treatment of cancer¹ and heavy metal poisoning², and in enzyme replacement therapy³. The advantages of a liposome drug over the use of free or polymer-bound agent are decreased toxicity and degradation, the use of smaller doses and the possibility of targeting the liposome towards a given tissue or site⁴. The localised administration of therapeutic agents, such as the intra-articular injection of cortisol esters in the treatment of rheumatoid arthritis⁵, is a situation where the use of liposomes as a means of providing a stable particulate suspension of entrapped steroid might be used to advantage. This method should reduce considerably the effective dose required to produce relief, and diminish side effects due to escape of steroid from the joint⁶. We report here that the treatment of an acute experimental arthritis in the rabbit with a liposome preparation containing cortisol results in a significant reduction in joint temperature and diameter, whereas treatment with an equivalent amount of cortisol alone, or with liposomes lacking cortisol, does not reduce these two parameters of inflammation.

Old English rabbits (body weight 1.5-2.0 kg) were used throughout the study. A bilateral inflammation of the knee joints was induced by the intra-articular injection of a complex formed from 7.5 mg poly-D-lysine (molecular weight 150,000) and 7.5 mg hyaluronic acid⁷ (both Miles). The initial inflammation induced by the complex is an acute synovitis with fibrin exudation and polymorphonuclear leukocyte infiltration. After 1 week this stage merges with a subacute phase showing macrophage infiltration. At 2-3 weeks after induction the arthritis is fully developed with lymphocytic aggregations, plasma cell infiltration, and the appearance of pannus. Evidence of active chronic inflammation then persists for 3-4 months, after which signs of increased fibrotic scarring occur. This process is normally complete within 7 months.

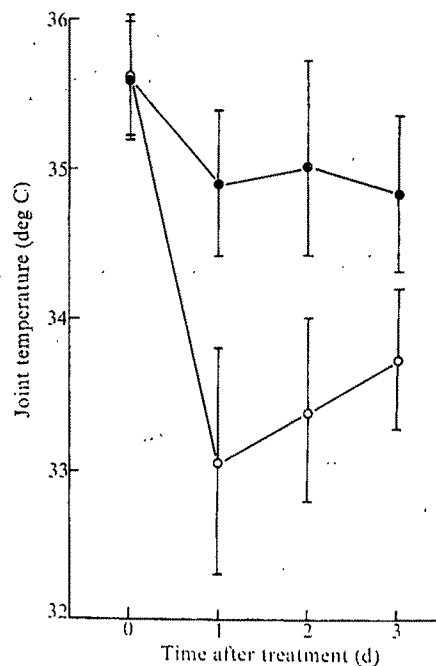
Infra-red radiation from the surface of the joint and its maximum diameter were used to follow the course of inflammation and to monitor the effect of treatment. The liposome

preparation containing cortisol palmitate was prepared as previously described⁸ using dipalmitoylphosphatidylcholine and egg yolk phosphatidic acid. Control liposome preparations were made by omitting the cortisol palmitate. Control liposomes, liposomes containing cortisol palmitate, or cortisol acetate particles in saline suspension were injected intra-articularly into one joint 4 d after induction of the inflammation. The effect of treatment on joint temperature and diameter was monitored for a period of 3 to 6 d.

Treatment of the experimental arthritis with a liposome preparation containing 35.5 µg cortisol palmitate (equivalent to 20 µg cortisol) resulted in a significant and sustained fall in the temperature (Fig. 1) and the diameter (Fig. 2) of the injected joint. The reduction in both these parameters of inflammation occurred within 24 h and there was no evidence of a contralateral effect in the uninjected joint. Neither injection of a liposome preparation containing 35.5 µg of the palmitate ester of 11- α -cortisol, a stereoisomer of cortisol which has no glucocorticoid activity⁹, nor a control liposome preparation containing no steroid had any effect on temperature or diameter. The injection of control liposomes, or liposomes containing cortisol palmitate into normal rabbit joints did not cause an increase in temperature or diameter, and it was concluded that the preparations were devoid of any inflammatory activity. There have been previous reports of liposomes having biological activity in their own right¹⁰, but the liposomes used in this study seemed to be without activity. The injection of cortisol acetate (equivalent to 20 µg cortisol) in saline suspension did not reduce the temperature or diameter of inflamed joints. Anti-inflammatory activity was seen at much higher doses (equivalent to 0.2-2.0 mg cortisol) and was restricted to a 24 h fall in temperature and diameter, whereas liposomes containing cortisol palmitate produced a sustained (3-5 d) reduction in these parameters of inflammation.

It was shown previously⁸ that cortisol esters are retained in liposomes prepared from dipalmitoylphosphatidylcholine and phosphatidic acid only if there is a hydrophobic anchor.

Fig. 1 Effect of liposomes containing cortisol palmitate on joint temperature. Hair was removed from the joint before the start of the experiment with a commercial depilatory cream. The surface temperature of an area 6 mm in diameter situated laterally over the joint space was measured using a Heimann KT 41 radiation thermometer. The joint surface temperature was allowed to equilibrate with the controlled ambient temperature ($20.5 \pm 0.25^\circ\text{C}$) before measurements were taken. Values are mean \pm s.e.m. of 6 animals. \circ , Treated joint; \bullet , untreated joint.



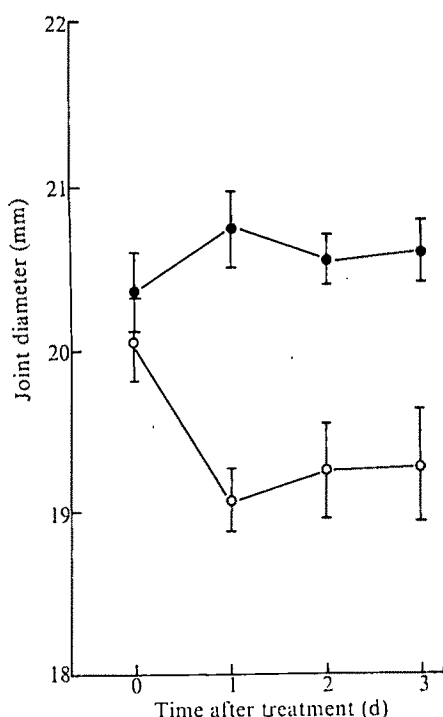


Fig. 2 Effect of liposomes containing cortisol palmitate on joint diameter. Maximum joint diameter was measured using a Baty spring-loaded micrometer. Values are mean \pm s.e.m. of 6 animals. O, Treated joint; ●, untreated joint.

The demonstration that an injection of cortisol palmitate incorporated into a liposome preparation has a much greater anti-inflammatory activity than an equivalent dose of microcrystalline cortisol acetate, a standard anti-inflammatory steroid ester, validates the use of liposome preparations for local delivery of steroids.

This work was carried out at Strangeways Research Laboratory, Cambridge, and at ICI Pharmaceuticals Division, Alderley Park, Cheshire. Similar results were obtained at both centres.

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Received 30 September; accepted 30 November 1977.

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Relationship between haptoglobin and *Streptococcus pyogenes* T4 antigens

DURING a survey of agglutinating activity of human saliva against streptococci¹ we found that saliva from individuals of blood group O agglutinated a particular strain of group G streptococci more often than did serum from those with blood group A or B. When we tested 833 sera we found a dimorphism for antibody-like substances against group G streptococci—sera could be divided into two groups according to their agglutination titre against group G streptococci. The first group had low titres, not rising above 1 : 4 and more usually 1 : 1; the second group had much higher titres ranging from 1 : 200 to 1 : 3,200. There was no overlap between the two groups of sera. We thought that this might be related to a genetic system, probably diallelic. We now report a relationship between the haptoglobin genotype of serum and the streptococcal antigens with which it reacts.

We tested our samples for a wide range of polymorphic blood groups (ABO, MNSs, P₁/P₂, rhesus, K/k, Le^a/Le^b, Duffy and Kidd), serum groups (Gm 1, Gm 2, Gm 4, Hp, Gc), enzyme groups (acP, PGM, ADA, AK, GPT and EsD) and HLA groups (factors of loci Sd¹ and Sd²). All sera without agglutinating activity or with low titres against a particular strain of group G streptococci were of the haptoglobin (Hp) type Hp 1-1. All sera with high titres, however, were of Hp types 2-1 or 2-2. There was no effect due to sex or age.

Further studies showed that sera with the Hp² gene product reacted with *Streptococcus pyogenes* strains carrying the T antigen complex T4/24. This antigen may also be present in some C and G group streptococci and it was only these strains that were agglutinated with high titres by Hp 2-2 and Hp 2-1 sera. In our series 1.4 % of group C streptococci and 6.4 % of group G streptococci carried the T4/24 antigen. A relationship between the T4/24 antigen of streptococci and haptoglobin types has not been described before.

The mechanism of the reaction is unknown but the reacting substance in all human sera is haptoglobin itself. This was demonstrated in cooperation with Dr Uhlenbruck in Cologne, using a purified haptoglobin preparation (98 % purity, Behringwerke, Marburg) which agglutinated the streptococci in the same way. Further evidence was given by a survey of human cord blood samples. If agglutination was negative, haptoglobin could not be detected. Animal sera with Hp 1-1-like patterns (rhesus monkey, rabbit, pig and sheep) did not agglutinate the T4/24 streptococci or did so only to low titres.

Finally, we found that the agglutinating activity of Hp 2-2 or Hp 2-1 sera could be inhibited by the addition of Hp 1-1 serum of human or animal origin before the test. We believe that Hp 1-1 protein represents a form of 'blocking antibody' for the appropriate streptococci.

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Partial γ l fragment is a 'natural' bovine IgG1 fragment without detectable class-specific γ determinants

IgG in bovine sera generally consists of a mixture of the two subclasses IgG1 and IgG2. While testing the reliability of the radial immunodiffusion (RID) method for quantifying IgG in bovine body fluids¹, we found a discrepancy. With most sera tested, the combined amounts of IgG1 and IgG2 determined using subclass-specific antisera was equal to the amount of IgG determined with class-specific antisera against γ determinants, that is common to IgG1 and IgG2. But with a few sera the amounts were not equal (unpublished results). (Class-specific antisera against γ determinants were produced by removing antibodies against subclass determinants on immunoabsorbent columns, starting from antisera which contained antibodies against both class ($=\gamma$) and subclass ($=\gamma$ 1 and/or γ 2) determinants.) Using immunoelectrophoretic (IE) analysis, we have now shown that those sera contain a 'fast' IgG component as well as both IgG subclasses. The third component was represented by an arc in the anodic region of the precipitation pattern, and it proved partially identical to that of IgG1 (Fig. 1). Such an arc can be seen in a photograph of IE patterns of sera from calves infected with *Trypanosoma vivax*, although the arc is not mentioned in the report².

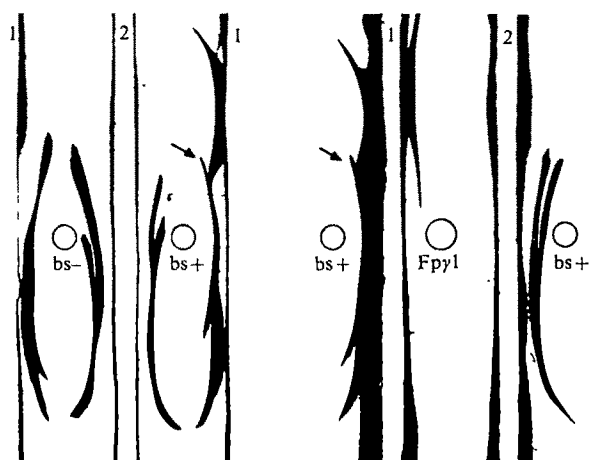


Fig. 1 bs+, bovine serum containing Fpyl (6 μ l); bs-, normal bovine serum (6 μ l); Fpyl, partial γ l fragment (24 μ l, 1 mg ml⁻¹); 1, goat anti-bovine IgG1; 2, goat anti-bovine IgG2. The anode is at the top. Arrows indicate reaction of partial identity of Fpyl and IgG1.

The arc in Fig. 1 was produced with antisera against IgG1 that contained antibodies against γ and γ l determinants. When we used antiserum against IgG2 that contained antibodies against L chain, γ 2 and γ determinants, the arc was absent (Fig. 1). The absence of γ determinants and the presence of γ l determinants on the proteins in this arc explained the discrepancy in RID values. The IE arc seemed to represent all or a part of a special kind of IgG1. Therefore, and for reasons explained below, this protein is referred to as partial γ l fragment (Fpyl).

Serum samples containing Fpyl were collected monthly from cows in connection with an investigation into the occurrence of parasitic infections in the field³. They were stored at -20 °C. It was unlikely that 'ageing' of sera gave rise to Fpyl because some freshly collected serum contained Fpyl whereas older serum from the same animals did not. Sometimes serum collected from the same animal in two successive months produced the IE arc, whereas samples collected shortly before and after this period contained little or no Fpyl.

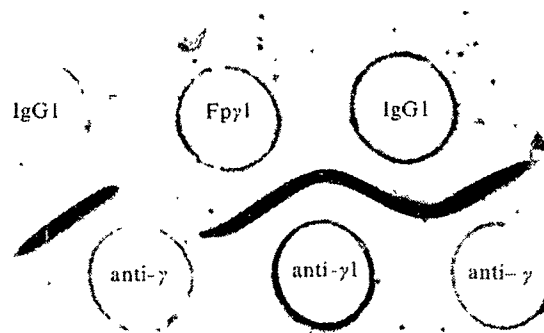
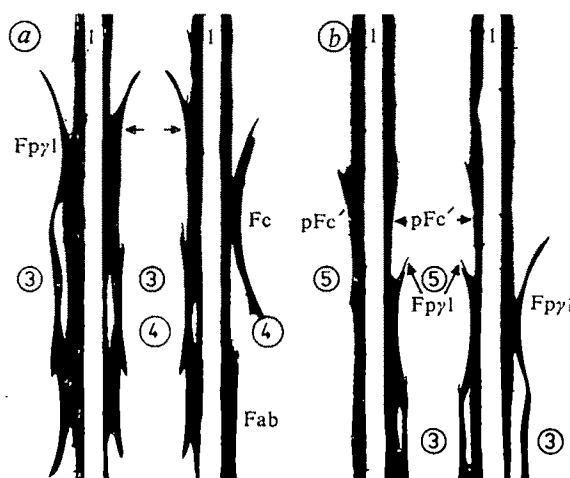


Fig. 2 The concentration of IgG1 was ± 0.1 mg ml⁻¹, and of Fpyl ± 1.0 mg ml⁻¹, anti- γ , goat antiserum specific for IgG class determinants; anti- γ l, goat antiserum specific for IgG1 subclass determinants. Note the reaction of complete identity of IgG1 and Fpyl in the reaction with antiserum specific for γ l determinants and the absence of the precipitation reaction of Fpyl with antiserum specific for γ determinants.

To characterise Fpyl, we isolated it on immunoabsorbent columns⁴ loaded with purified antibodies against γ l determinants (column 1) and γ determinants (column 2). Serum samples containing Fpyl were applied to column 1 and the mixture of Fpyl and IgG1 was eluted with 3M ammonium thiocyanate in phosphate-buffered saline. When this mixture was applied to column 2, only IgG1 was bound.

The protein thus isolated produced an IE arc comparable with the Fpyl arc from the original serum (Fig. 1). In double immunodiffusion (DID), with antiserum specific for γ l determinants Fpyl gave a reaction of complete identity to IgG1 but did not react with four class-specific antisera specific for γ determinants, although these reacted with IgG1. The reaction with one of these antisera is shown in Fig. 2. The isoelectric point of Fpyl, determined by isoelectric focusing⁵, was between 4.4 and 4.7. Estimates of the molecular weight of Fpyl, obtained by gel-filtration using Sephadex G-200 Fine⁶ and electrophoresis in polyacrylamide gel⁷ without reducing agents were 42,000 and 44,000

Fig. 3 Double immunoelectrophoresis patterns of Fpyl with Fc fragment (a) and pFc' fragment (b). The anode is at the top. Note the absence of Fc spurs at the inner side of the Fpyl arcs (arrows in a) and the presence of Fpyl spurs at the inner side of the pFc' arcs, indicating reactions of complete and partial identity, respectively. The splitting of the Fpyl arcs at the inner side of pFc' arcs is brought about by the presence of the pFc' arcs, preventing antibodies against determinants common to pFc' and Fpyl from reaching Fpyl. 1, Goat antiserum against IgG1; 3, bovine serum containing Fpyl; 4, Fc/Fab mixture; 5, pFc'.



respectively. Reduction with 0.1% dithioerythritol, which disassociates the H and L chains of IgG1 (molecular weight 58,000 and 23,500, respectively in polyacrylamide gel) did not affect the value of 44,000 for Fpy1. Therefore we assumed that Fpy1 consists of one polypeptide chain. On the basis of the estimates of molecular weight of Fpy1; the presence of $\gamma 1$ determinants; the absence of detectable L chain determinants, and a decreased concentration of IgG1 in sera containing Fpy1 (see below), we assumed that Fpy1 emanated from an H chain of IgG1. Double immunoelectrophoretic analysis⁸ showed complete identity between Fpy1 and Fc fragment (obtained after digestion of IgG1 with papain⁹) and partial identity between Fpy1 and pFc' fragment (obtained after digestion of IgG1 with pepsin¹⁰) (Fig. 3).

The pFc' fragment includes the complete COOH-terminal part of the H chain (CH3 region) and the Fc fragment approximately covers the combined CH2 and CH3 regions. The molecular weight of Fpy1 (42,000–44,000) accounts for about three-quarters of the estimated molecular weight of an H chain. This molecular weight (58,000) is in fair agreement with estimates of molecular weights of bovine IgG1 H chains reported before (for example, ref. 11). Although the precise location of Fpy1 in the H chain remains to be determined (a small fragment may be missing from one or both sides of the proposed polypeptide chain) Fpy1 probably consists of the combined CH1, CH2 and CH3

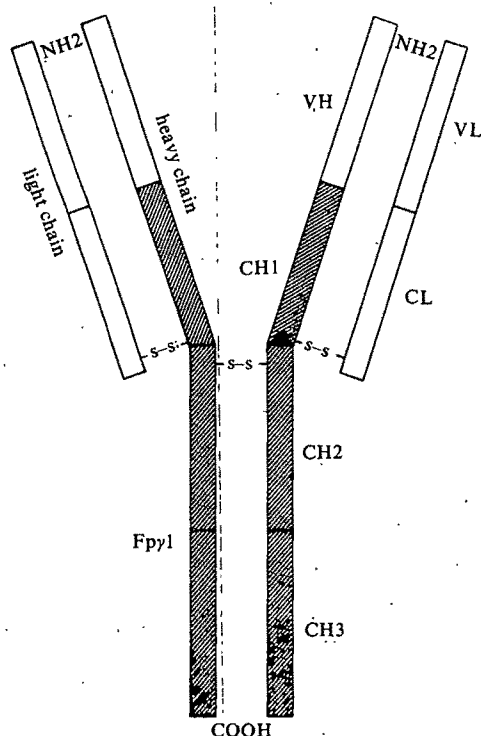


Fig. 4 Schematic drawing of the linear structure of IgG1, with an indication of variable (V) and constant (C) regions and the probable position of the Fpy1-fragment (hatched parts).

regions of the heavy chains of the IgG1 molecule (Fig. 4). One may argue instead that part or all of the VH region forms part of Fpy1, so that there are deletions in the H chains as in heavy-chain disease proteins¹². This is unlikely in view of the presence of the CH2 and CH3 regions in Fpy1 and because sera collected shortly before and during the appearance of Fpy1 always contained IgG1 with H chains of normal molecular weight. We assume that this IgG1 is the source of Fpy1, for the concentration of IgG1 is strongly reduced in sera containing Fpy1.

It is surprising that class-specific γ determinants cannot be detected on Fpy1 with either precipitation techniques (IE and DID) or immunoadsorbents. Perhaps γ determinants are more labile than $\gamma 1$ determinants, or they are on the Fd part of IgG1 nearer to the VH/CH1 boundary than $\gamma 1$ determinants, or near interchain disulphide bonds.

We have not detected Fpy1 in hundreds of normal sera, in spite of a supposed continuous catabolism of antibodies directed against various antigens. But Fpy1 appears in sera of cows intentionally infected with *Fasciola hepatica*, and it is likely that the sera in which Fpy1 was found originally were from similarly infected cows. Our results suggest that while antibodies are being bound to antigens of parasitic origin, combined CH1, CH2 and CH3 regions of $\gamma 1$ chains, and presumably CL regions as well, are separated from the rest of the IgG1 molecule, breaking disulphide bonds. Whether CL regions remain intact for some time and appear in Fpy1-positive serum is not known.

The chance of detecting proteins consisting of CL regions only is small in view of the short half-life of light chains¹³. We assume that Fpy1 is generated while the immunological system is confronted with a large antigenic load, leading to, for example, incomplete phagocytosis of the antigen-antibody complexes. It remains to be seen whether Fpy1 arises only in response to parasitic or other corpuscular antigens or whether large amounts of any antigen lead to comparable fragmentation of IgG1. Although fragmentation has been described for Fc- and Fab-like fragments in normal urine¹⁴ and plasma¹⁵, Fpy1 seems to be the first reported catabolic fragment of Ig that is probably related to antigen-antibody interactions *in vivo*.

I thank Mrs Jacqueline J. L. Noordijk and Mrs Willy Stok for technical assistance and Dr L. J. van Tiggele for bovine sera.

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Received 26 September; accepted 25 November 1977.

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Production of HLA antibody in mice immunised with syngeneic mouse-human hybrid cells containing human chromosome 6

XENOIMMUNISATION with human cells or isolated molecules found on the surface of human cells results in the production of antibodies to a variety of human alloantigens (refs 1–3 and unpublished results). The genetic loci which specify the antigens being recognised can then be determined by using rodent-human hybrids containing various human chromosomes as target cells. In this manner, chromosome 11 has been found to include genes coding for a series of antigens designated A_L (refs 1, 4) or HSA-1 (ref. 2) and chromosome 15 to code for β_2 microglobulin⁵. Murine-human somatic cell hybrids with one or a limited number of human chromosomes have been used by us and by others to obtain antisera in mice syngeneic to the mouse parental cells reactive with antigens coded for by

specific human chromosomes^{2,6-7}. The advantage of this method is that antisera generated are specific for the antigen(s) coded for by a particular human chromosome and the reactivity is to a limited number of human cell surface antigens. This allows for both systematic genetic mapping of these antigens and detection of previously unknown cell surface molecules. Chromosome 6 has been shown both by pedigree analysis⁸ and by the use of interspecific somatic cell hybrid target cells⁹ to be the location of the genes coding for the serologically defined antigens (HLA) of the major histocompatibility complex (MHC). We have used a human-mouse somatic cell hybrid containing several human chromosomes, including chromosome 6, to immunise mice syngeneic to the mouse parental cell. The reactivity of the absorbed antiserum has been analysed both serologically and by sodium dodecyl sulphate polyacrylamide gel electrophoresis after immunoprecipitation. Evidence is presented here to show that mice immunised with somatic cell hybrids containing human chromosome 6 produce antibody which reacts with HLA as well as with other human lymphocyte cell surface antigens.

Hybrid cells used for immunisation were the Nude 1 (N 1) cell line¹⁰. This hybrid line was derived from a tumour grown in an athymic mouse injected with hybrid cells obtained by the Sendai virus-mediated fusion of C57B1/6 mouse peritoneal macrophages and LN-SV (an SV40-transformed human cell line derived from skin fibroblasts of a Lesch-Nyhan syndrome patient)¹¹. N 1 was found by isozyme analysis and karyotypic evaluation to contain human chromosomes 6, 7, 14 and 21. Clones containing chromosomes 7, 14 and 21 but not 6 have also been derived from this original hybrid.

The HLA type of human parental cell LN-SV was determined by the standard NIH microcytotoxicity assay in Terasaki plates. These cells were found to express HLA-A2 and HLA-B5 antigens. Clonally derived hybrid cells containing one copy of human chromosome 6 were used to absorb several cytotoxic HLA typing sera, specific for HLA-A2 and HLA-B5. These sera were then tested on a panel of human lymphocytes of known HLA type. Table 1 shows that the hybrid cells containing human chromosome 6 expressed HLA-A2, and were HLA-B5-negative. Hybrid cells without human chromosome 6 (human chromosomes 7, 14, and 21 present) did not absorb out HLA reactivity.

To determine if the murine humoral response to somatic cell hybrids containing human chromosome 6 is to MHC-defined antigens and/or to species-defined antigens, C57B1/6J mice were immunised with N 1 hybrid cells. At 7-10 d after each intraperitoneal injection of $1-3 \times 10^7$ trypsinised cells, mice were bled, and serum was separated and stored at -70°C until use. For these studies, anti-N 1 serum from the sixth immunisation was absorbed with another human-mouse

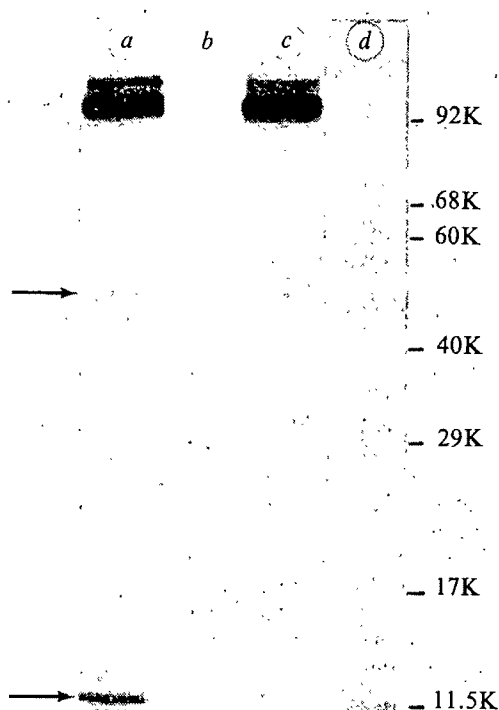


Fig. 1 Immunoprecipitation of ^{125}I -labelled surface proteins from human lymphocytes with absorbed anti-N 1 serum. Human peripheral blood lymphocytes (isolated from interface of Ficoll-Hypaque gradient (density 1.077) and washed three times with Dulbecco's modified PBS without Ca^{2+} and Mg^{2+} salts) were iodinated (New England Nuclear, NEZ-033H) by the lactoperoxidase-glucose oxidase method as previously described^{12,17}. Extracts were prepared by 1 h incubation (0°C) in 0.1% NP-40, 0.1 M Tris, pH 6.8, 15% glycerol and 2 mM phenyl-methylsulphonyl fluoride (PMSF). Iodinated extracts were incubated for 1 h, 0°C , at a 2:1 ratio with normal human serum from the donor of the lymphocytes or with rabbit anti-human β_2 microglobulin. Immune complexes were cleared from the samples by incubation at 0°C for 1 h with protein A-bearing *Staphylococcus aureus* Cowan 1 strain. Specific serum at a 1:5 final dilution was added to the precleared samples. After incubation at 0°C for 1 h, the immune complexes were absorbed to *S. aureus*, centrifuged and washed. After elution of the absorbed proteins from the bacterial surface (boiling *S. aureus* in 0.1 M Tris, pH 6.8, 15% glycerol, 2 mM PMSF, 2% sodium dodecyl sulphate (SDS) and 1 mM dithiothreitol) the bacteria were removed by centrifugation and the samples applied to 10% SDS-acrylamide gels with 5% stacking gels¹³. Gels were dried onto filter paper and exposed to Kodak NS2T X-ray film. Molecular weight markers for gel calibrations: cytochrome *c* (11,500), myoglobin (17,000), carbonic anhydrase (29,000), aldolase (40,000), catalase (60,000), bovine serum albumin (68,000), phosphorylase A (92,000); a, absorbed anti-N 1 serum, sample precleared with normal human serum; b, normal mouse serum, sample precleared with normal human serum; c, absorbed anti-N 1 serum, sample precleared with rabbit anti-human β_2 microglobulin; d, rabbit anti-human β_2 microglobulin serum, sample precleared with normal human serum.

Table 1 HLA expression on hybrid cells

HLA alloantisera specificity*	Absorbing hybrid	Human chromosome	HLA of lymphocytes A1, 2; B8	A2,29; B5, 12
A2 ^a	C1 35	+	0†	0
A2 ^a	C1 37	—	4†	4+
A2 ^b	C1 35	+	0	0
A2 ^b	C1 37	—	4+	4+
B5 ^c	C1 35	+	NR‡	3+
B5 ^c	C1 37	—	NR	3+
A2B5 ^d	C1 35	+	0	4+
A2B5 ^d	C1 37	—	3+	4+
A1B8 ^e	C1 35	+	4+	NR
A1B8 ^e	C1 37	—	3+	NR

*Sera obtained from Transplantation Immunology Branch, National Institute of Allergy and Infectious Diseases, National Institutes of Health: a, 2-50-6-03-15-03; b, 1-01-7-02-07-02; c, 2-52-1-01-14-01; d, 2-58-9-02-21-04; e, 1-04-1-02-15-01.

†0-4+, strength of cytotoxic reaction; 0, 0-10%; 1+, 10-30%; 2+, 30-60%; 3+, 60-80%; 4+, 80-100%.

‡No reaction; lymphocytes do not express this antigen.

hybrid cell line of the same origin containing human chromosomes 7, 14 and 21. Testing revealed that the absorbed anti-N1 serum reacted only with hybrids containing chromosome 6.

To define the cell surface molecules with which the absorbed anti-N 1 serum reacted, we prepared extracts of human peripheral blood lymphocytes which had been enzymatically labelled with ^{125}I using the lactoperoxidase-glucose oxidase method¹². The extracts were reacted with absorbed anti-N 1 serum, or normal mouse serum, and the immune complexes were analysed on 10% polyacrylamide gels¹³. As shown in Fig. 1a, reaction of lysates with absorbed anti-N 1 serum results in precipitation of several proteins; two of these (see arrows) have molecular weights consistent with those of HLA and its associated light chain, β_2 microglobulin¹⁴. No radio-labelled bands are seen when normal mouse serum is used (Fig.

1b). When the extracts were pre-cleared by treatment with rabbit anti-human β_2 microglobulin serum (Dakoimmunoglobulin; Copenhagen, Denmark) (see Fig. 1 legend) and then reacted with anti-N 1 serum, the 47,000 molecular weight (MW) and 12,000 MW bands disappeared, leaving the higher molecular weight proteins coded for by human chromosome 6 (Fig. 1c). Electrophoresis of immune complexes of anti-human β_2 microglobulin serum with the labelled human lymphocytes shows β_2 microglobulin, the 12,000 MW band, and the co-precipitated HLA (Fig. 1d).

Further evidence that the reactivity is to HLA is provided by lysostrip experiments shown in Table 2 (procedure and theory described in refs 15 and 16). Normal human lymphocytes of known HLA specificities after reaction with anti-N 1 are incubated with goat anti-mouse IgG. The cells are then reacted with HLA typing sera. The fact that reactivity to each HLA specificity is affected by this stripping procedure provides evidence that anti-N 1 serum is reacting with HLA molecules on the lymphocyte surface. It is important to note that although HLA-A2 is the only specificity shown to be present on the immunising cell surface, both HLA-A and HLA-B antigens are affected by the stripping procedure. This has been observed with antisera and lymphocytes of other HLA types (data not given). The response seems to be either to a common determinant of HLA molecules or a multiclonal response to many portions of the HLA molecule. If some B cell clones were responding to the private HLA specificities present on the immunising hybrid, they would not be detected because of the overwhelming response to other portions of the molecule. Immunising mice with hybrids containing human chromosome 6 and hybridising B cells from these mice to myeloma cells may result in the constitutive production of monoclonal antibodies specific for the HLA allele(s) of the immunising cell. *In vitro* xenoimmunisation of both human¹⁷ and rat¹⁸ cells with mouse lymphoid cells results in the development of cytotoxic effector cells which are specific for the immunising H2 haplotype. It seems that limited clonal stimulation occurs with the major reactivity limited to the recognition of the allelic specificities of H2.

Table 2 Susceptibility of human lymphocytes to cytotoxic killing after lysostripping with anti-N 1 and goat anti-mouse IgG treatment

Antiserum tested with*	% killed†
N1	7
HLA-A1 ^a	24
HLA-A2 ^b	1
HLA-A2 ^c	28
HLA-B8 ^d	32
HLA-B8 ^e	10
Human‡	100

2.5×10^5 Ficoll-Hypaque-separated human peripheral blood lymphocytes (HLA-A1, 2; B8) were treated with absorbed anti-N 1 serum (or normal mouse serum as control) 1:20, 30 min, 4 °C; treated with goat anti-mouse IgG, 1:5; 30 min, 4 °C, incubated 30 min., 37 °C; treated with goat anti-mouse IgG, 1:5, 30 min, 4 °C; washed three times between each treatment incubated 30 min, 37 °C, and washed twice. Volumes were kept constant at 100 μ l. All washes were with 1 mM HEPES-buffered RPMI 1640 containing 10% foetal bovine serum. Antisera were diluted in 1 mM HEPES-buffered RPMI 1640. This procedure was to 'induce' resistance to antibody-mediated complement-dependent cytotoxicity for the antigen(s) recognised by the anti-N 1 serum. The 'lysostripped' or treated control cells were then tested in an antibody-dependent complement-mediated microcytotoxicity assay to determine whether the cells were resistant to lysis by the indicated sera. (For procedure and discussion of theory, see refs 15 and 16.)

*Sera from Transplantation Immunology Branch, National Institute of Allergy and Infectious Diseases, National Institutes of Health: a, 2-65-9-11-17-08; b, 2-50-6-03-15-03; c, 1-01-7-02-07-02; d, 2-50-6-10-04-01; e, 2-51-2-04-27-01.

†Calculated as follows:

$$\frac{(\% \text{ anti-N 1 treated dead} - \% \text{ c' control dead})}{\% \text{ normal serum treated dead} - \% \text{ c' control dead}} \times 100$$

The test sera used after lysostrip treatment were used at a dilution that killed >60% of the normal serum control; in most cases 80–90% of control cells were killed at the dilution selected.

‡Serum from C57Bl/6 mice immunised with LN-SV human cells.

We suggest that human-mouse hybrid cells containing human chromosome 6 can be used to immunise mice to produce antibodies to the major histocompatibility complex. Additional cell surface molecules coded for by human chromosome 6 are detected with the anti-N 1 sera.

This work was supported, in part, by a Career Development Award from the US NCI and grants from the NCI, American Cancer Society, National Foundation-March of Dimes, and a NSF Fellowship (R.M.).

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Received 11 October; accepted 21 November 1977.

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Lymphocyte induced stimulation of human chorionic gonadotrophin production by trophoblastic cells *in vitro*

STIMULATION of target cells following an immunological interaction has been shown to occur under suitable conditions. For example, B lymphocytes undergo blast transformation when cultured in the presence of low levels of anti-immunoglobulin¹, and likewise thyroid cells can be stimulated by LATS (long acting thyroid stimulator) and LATS protector^{2,3}, both of which are immunoglobulins, resulting in increased thyroxine production. There is no evidence that target cells can be stimulated in such a manner after contact with lymphocytes. We present here evidence that increased chorionic gonadotrophin (HCG) production results when trophoblasts are cultured in the presence of maternal lymphocytes.

Trophoblastic cells were obtained from trypsinised fresh placentae and incubated for 1–3 d in Eagle's Dulbecco Modified Medium with 10% foetal calf serum in the presence or absence of lymphocytes. Lymphocytes were obtained from either normal donors, from patients with gynaecological cancer, or from pregnant women at delivery. The lymphocytes were separated on Hypaque-Ficoll and added to the trophoblast cell cultures in the ratio of 1:1 or 5:1. Production of HCG by the cultures was assessed by a radioimmunoassay technique as performed in the section of biochemistry of this hospital.

When trophoblast cells were cultured alone, there was a detectable increase in HCG production after 24 h, followed by a continuous increase over the first 3 d (Table 1). When lymphocytes from normal donors were added to trophoblast cells at a ratio of 5:1 there was slight reduction of hormone production, significant after 3 d ($P < 0.01$, Student *t* test). When lymphocytes from patients with gynaecological cancer were

Table 1 % Increase in HCG production by trophoblast cells cultured in the presence of lymphocytes (mean \pm s.e.m.).

	No. of cultures	Time (Days)			
		0	1	2	3
Trophoblast only	28	100	143.4 \pm 12.9	209.3 \pm 9.6	248.6 \pm 8.7
Trophoblast + normal lymphocytes 5:1	14	100	175.5 \pm 19.4	189.6 \pm 6.1	193.7 \pm 10.3
Trophoblast + maternal lymphocytes 5:1	13	100	164 \pm 8.9	236.5 \pm 7.5	316.5 \pm 16.5
Trophoblast + maternal lymphocytes 1:1	4	100	159.9 \pm 12.8	191.6 \pm 18.7	—
Trophoblast + cancer lymphocytes 5:1	10	100	156.6 \pm 17.5	190.2 \pm 20.3	185 \pm 10
Trophoblast + unrelated maternal lymphocytes 5:1	2	100	—	188	—

added to the trophoblastic cells there was also significant reduction in hormone production only after 3 d ($P < 0.01$). When maternal lymphocytes taken at the time of delivery by Caesarean section were added to the trophoblastic cells at a ratio of 1:1 there was no significant difference, but when the ratio was 5:1 there was a significant increase in the amount of hormone produced after 2d ($P < 0.05$) and 3 d ($P < 0.01$). Comparison of the values obtained from cultures with added normal lymphocytes and those of cultures with added maternal lymphocytes shows a high degree of difference after 2 and 3 d ($P < 0.001$). No stimulation occurred when maternal lymphocytes from unrelated donors were used.

The reduction of HCG production by trophoblastic cells after culture with allogeneic lymphocytes is consistent with the findings of Currie⁴, Douthwaite and Urbach⁵, and Taylor and Hancock⁶, who described lysis of trophoblastic cells by allogeneic leukocytes. This 'allogeneic inhibition' occurs after 24–48 h in culture, and pre-sensitisation of the lymphocytes by target-type antigens is not required. The stimulation of HCG production by maternal lymphocytes is more difficult to explain. This phenomenon may be equivalent to stimulation of target cells by immunoglobulin, as suggested by Rose⁷ for thyroid cell stimulation by lymphoid cells. Further studies are in progress in order to establish whether this phenomenon is in any way altered in abnormal pregnancy, such as pre-eclampsia.

We thank the obstetricians and nursing staff of the Royal Women's Hospital, Melbourne, for placenta and blood samples, and Dr I. Horacek and Staff, Section of Biochemistry of this Hospital, for help with the radioimmunoassay for HCG.

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Electrophoretic mobility as a sensitive probe of lectin-lymphocyte interaction

TREATMENT of murine T lymphocytes with concanavalin A (con A) at concentrations as low as 10^{-17} g ml⁻¹ produces significant increases in their electrophoretic mobility. The effect is blocked by α -methyl-D-mannopyranoside (α -MM). Phytohaemagglutinin (PHA) produces similar changes in T-cell mobility while treatment with lipopolysaccharide (LPS), a

B-lymphocyte mitogen, does not. We present here evidence indicating that the altered mobility of con A-treated T cells is due to the rapid release of a factor from the few cells reacting directly with the lectin.

The blast transformation of lymphocytes resulting from treatment with lectins such as con A is of considerable interest since it may serve as a model for the events which occur when lymphocytes are specifically stimulated by antigens. Lectin-induced capping, increased ³H-thymidine uptake and development of blast morphology are relatively slow phenomena. Since the earliest events associated with lymphocyte activation probably occur on the cell membrane within minutes, a technique that would allow immediate assessment of membrane alterations would be extremely useful.

Cell electrophoresis provides a probe of membrane interactions involving changes in the surface charge density, these changes being reflected by an alteration of the cell's electrophoretic mobility¹. Cell electrophoresis has previously been used as a preparative method for separating T and B lymphocyte populations^{2–6}, as a probe for lymphocyte membrane chemical composition⁷ and for studying lymphocyte interaction with a variety of chemical agents including lectins^{8–10}, polynucleotides¹¹, and antisera^{12,13}.

In view of the probable conformational changes in the lymphocyte membrane associated with transformation, it has been suspected that changes in the charge density associated with the membrane would result in an alteration in the electrophoretic mobility of the cell. We have found that extremely low concentrations of con A induce significant increases in the electrophoretic mobility of murine T cells. Moreover, on exposure to con A there is apparently a rapid release of a factor from some murine T cells which induces increased mobility in the remainder of the cells.

Electrophoretic measurements were performed using the technique of Seaman *et al.*¹ in which a suspension of cells in a controlled potential gradient is directly observed through a microscope. The results are expressed in $\mu\text{m s}^{-1}\text{V}^{-1}\text{cm} \pm 1\text{s.e.m.}$ and are based on the time of migration of a series of cells across a fixed number of divisions of an eyepiece graticule. All mobility measurements were carried out at 25 °C in 0.01 M phosphate-buffered saline, pH 7.2, using a microinjection technique to introduce cell suspensions (20–30 μl) into the observation region of the electrophoresis chamber. In most experiments, measurements were collected for 20 different cells, with a reversal of the polarity of the field between measurements. Mobility values of human and murine erythrocytes were -1.08 ± 0.02 and $1.05 \pm 0.02 \mu\text{m s}^{-1}\text{V}^{-1}\text{cm}$ which is in agreement with values reported by others^{1,14}.

Ether-anaesthetised LAF₁ mice (Jackson Laboratories) were killed and their spleens removed. Cell suspensions were prepared and washed three times in 0.01 M phosphate-buffered saline containing 5% heat-inactivated foetal calf serum (PBS-FCS), pH 7.2, by centrifugation at 490 g for 10 min at 4 °C. An enriched T-lymphocyte suspension was prepared by the method of Julius *et al.*¹⁵ using nylon wool columns (Leuko-Pak Leuko-cell Filters, Fenwal Laboratories).

Con A (Miles-Yeda) was obtained as a three-times re-

crystallised powder and a stock solution ($100 \mu\text{g ml}^{-1}$) was prepared by weighing out 10 mg of con A and diluting to 100 ml with PBS. Stock con A solutions were stored at -20°C in 1 ml aliquots. Additional dilutions of con A were made by serial dilutions in PBS. α -Methyl-D-mannopyranoside (Sigma) was dissolved in PBS to yield a concentration of 0.2 M.

Phytohaemagglutinin-P (PHA) and *Salmonella typhimurium* lipopolysaccharide (LPS) (both Difco) were dissolved in distilled water. Stock solutions of PHA (0.1 ml) and LPS ($100 \mu\text{g ml}^{-1}$) were kept frozen at -20°C .

Each experiment was performed by mixing 100 μl of lectin solution or PBS and 100 μl of cell suspension in a test tube and incubating at 37°C for 5 min. Reaction mixtures containing α -methyl mannoside (α -MM) were prepared by adding 100 μl of cells to a mixture of 50 μl of 0.2 M α -MM and 50 μl of lectin solution. In experiments where PHA or LPS were tested, the stock PHA and LPS solutions were diluted with PBS to a concentration of 1 : 1000 and $0.25 \mu\text{g ml}^{-1}$ respectively.

Our initial experiments were performed using con A at final concentrations between 0.1 and $5 \mu\text{g ml}^{-1}$. A significant increase was observed (Fig. 1) in the mobility of T cells treated with con A compared with untreated T cells.

To determine whether this effect depends on con A binding to the lectin-specific carbohydrate moieties on the membrane, we repeated the experiments adding α -MM to the reaction mixture at a final concentration of 0.05 M. This sugar completely eliminated the con A-induced mobility change (Fig. 1). We excluded the possibility that the mere presence of con A in the supporting electrolyte (in contradistinction to its effect on the membrane) led to the changes in mobility. Following incubation of the T cells with con A $0.1 \mu\text{g ml}^{-1}$, we centrifuged the incubation mixture, removed the supernate and resuspended the cells in PBS. The observed mobility of -1.37 agreed well with that of the cells in the presence of the same concentration of con A and suggested that the con A effect is indeed attributable to its binding to the membrane or to induced changes in the membrane. Mitogen-induced increases in the mobility of T cells was also observed with a 1 : 1000 dilution of PHA (-1.35 ± 0.03). However, the B cell mitogen LPS, at a concentration that induces lymphocyte transformation of B cells ($0.05 \mu\text{g ml}^{-1}$) failed to induce a significant change in the mobility (-1.07 ± 0.04) of T cells.

At con A concentrations greater than $5 \mu\text{g ml}^{-1}$, a dose-dependent decrease in T-cell mobility was observed (Fig. 1). This is consistent with observations made in high concentration ranges by other investigators⁸⁻¹⁰. It can be seen (Fig. 1) that at concentrations of 25 and $50 \mu\text{g ml}^{-1}$, the T-cell mobility was actually less than that of the untreated cells. This can be interpreted as the consequence of the physical presence of large numbers of molecules of con A on the cell surface. The fact that at a con A concentration of $25 \mu\text{g ml}^{-1}$, α -MM returned the mobility to untreated levels (Fig. 1) supports this hypothesis.

Fig. 1 Influence of con A on the electrophoretic mobility of murine T lymphocytes. White bars refer to cell mobility in the absence of α -MM and black bars refer to cell mobility in the presence of α -MM. The standard error of the mean is given for the number of measurements indicated above each bar.

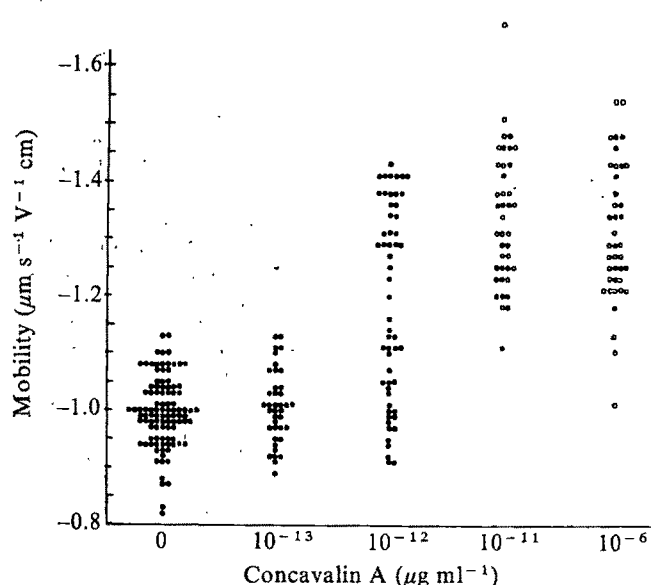
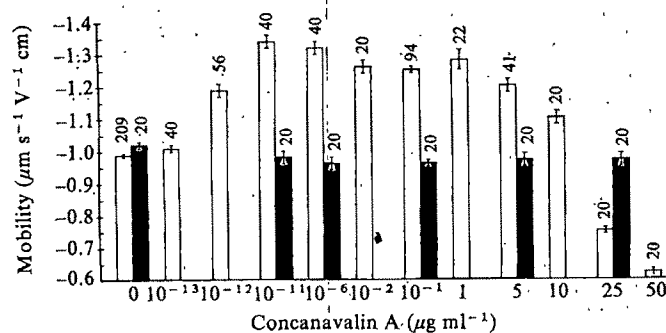


Fig. 2 Distributions of mobilities of individual cells treated with various concentrations of con A. Note that at the threshold concentration of $10^{-12} \mu\text{g ml}^{-1}$ of con A the mobility values of individual cells are distributed from control values to the maximum response values.

Furthermore, far from contradicting the results obtained at low concentrations, these data suggest that the increased mobility observed in those experiments can indeed be attributed to induced alteration of the membrane as opposed to the simple presence of a foreign molecule on it. The remaining experiments dealt with this hypothesis.

Decreasing concentrations of con A were incubated with T cells and mobility changes were clearly evident down to a concentration of $10^{-11} \mu\text{g ml}^{-1}$ (Fig. 1). At $10^{-11} \mu\text{g ml}^{-1}$ a maximal increase in electrophoretic mobility was observed with indications that the bulk of the cell population was responding, that is, on observation of the cell population in the electrophoresis apparatus there was no evidence of heterogeneity in the electrophoretic properties of the population. However, at $10^{-12} \mu\text{g ml}^{-1}$ a smaller increase in the mean mobility of the population was observed (-1.19 ± 0.02) and electrophoretic heterogeneity in the population was apparent (Fig. 2). The limited mobility distribution data collected suggested that this lower mean mobility value represented the composite of two cell populations: one consisting of cells with increased mobility and a second characteristic of unaffected cells (Fig. 2). Concentrations of con A below $10^{-12} \mu\text{g ml}^{-1}$ do not induce changes in the mobility of T cells.

The general T cell population response at the threshold con A concentration of $10^{-11} \mu\text{g ml}^{-1}$ was difficult to explain in terms of a direct interaction between lectin molecules and the surface of each lymphocyte in the population. Even if we assume the con A to exist as a monomer having a molecular weight of 27,000, at a threshold concentration of $10^{-11} \mu\text{g ml}^{-1}$, only 220 molecules of con A are necessary to initiate the observed mobility changes in 10^6 T cells. The short incubation time and the few molecules of con A needed to initiate the observed mobility changes suggested that factors distinct from con A binding led to the general T cell response. In order to determine whether con A binding of cells releases a soluble factor(s) which influences the mobility of non-con A binding cells, the following experiment was carried out.

Con A ($10^{-6} \mu\text{g ml}^{-1}$) was incubated with 10^6 T cells for 5 min at 37°C . The cell suspension was centrifuged at 1,000 g for 5 min at room temperature and 50 μl of the supernate was mixed with 50 μl of α -MM (final concentration 0.05 M) and 100 μl of untreated T cells (10^6) was added. This mixture was incubated for 5 min at 37°C and the mean mobility of the cells was -1.30 ± 0.02 . Supernate obtained from $10^{-6} \mu\text{g ml}^{-1}$ con A incubated without T cells for 5 min at 37°C , centrifuged

at 1,000g for 5 min, and added to 10^6 untreated cells in the presence of α -MM had a mean mobility of -1.00 ± 0.02 .

These observations indicate that con A treatment of T cells rapidly induces the release of a factor which can produce an electrophoretic mobility increase in the murine T cell population. Since electrophoresis is sensitive to the charge density on the cell surface, a marked alteration in the cell surface organisation of the T lymphocytes is indicated. Frelinger *et al.*¹⁶ reported that only a small portion of T cells directly respond to con A. They demonstrated that the con A responding T cells were Ia positive and release a factor that can promote the mitogenic response of Ia-negative T cells.

Studies are in progress to clarify the relationship between the factor which induces changes in T-cell mobility and the mitogenic-promoting factor described by Frelinger *et al.*¹⁶ and to establish whether similar electrophoretic responses and factor(s) occur during the interaction of immune T cells with antigens.

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Amphetamine induces β -adrenergic receptor supersensitivity

THE interactions of amphetamines and catecholamines in the peripheral sympathetic nervous system and in the brain have been widely studied. Amphetamines have been reported to mimic catecholamines at their receptor sites¹⁻³, inhibit monoamine oxidase⁴, impair reuptake mechanism for the catecholamines⁵ and to directly release catecholamines into the synaptic cleft⁶. Although the consensus of the available literature indicates that *d*-amphetamine indirectly stimulates catecholamine postsynaptic receptors by increasing the release and blocking the reuptake of catecholamines^{7,8}, the question whether dopamine, noradrenaline or both, are of importance for the central actions of *d*-amphetamine is controversial^{7,8}. Recently, α -adrenergic⁹, β -adrenergic¹⁰⁻¹³ and dopaminergic^{14,15} receptors in brain tissue have been successfully identified by measuring the binding of radiolabelled ligands to specific receptor sites. Availability of such methods has permitted the examination of the effects of acute and chronic administration of psychotropic drugs on the postsynaptic catecholaminergic receptors in brain tissue^{13,16}. We report here the effects of acute and chronic

administration of *d*-amphetamine on the postsynaptic β -adrenergic receptors in rat brain.

Specific binding of ^3H -dihydroalprenolol to the brain particulate fractions obtained from control rats and from rats treated with a single dose of *d*-amphetamine (10 mg per kg body weight) was measured. The binding of ^3H -dihydroalprenolol to specific β -adrenergic receptors¹⁰⁻¹³, did not significantly change at 1, 12, 24, 48 and 96 h after administration of a single dose of *d*-amphetamine (results not shown). Thus, acute treatment with *d*-amphetamine did not significantly alter the sensitivity of β -adrenergic receptors in rat brain. To evaluate the effects of chronic treatment with *d*-amphetamine on rat brain β -adrenergic receptors, specific binding of ^3H -dihydroalprenolol was measured 1, 12, 24, 48 and 96 h after the last dose of the drug. Although increased specific binding of ^3H -dihydroalprenolol to rat brain particulate fraction could be seen 1 h after discontinuation of chronic treatment, the increase was not significant when compared to that of control (Fig. 1). Maximal activation of β -adrenergic receptor binding was observed 12 h after cessation of *d*-amphetamine injection. Specific binding of ^3H -dihydroalprenolol at 12, 24, and 48 h after the last injection was significantly greater than that observed in the control preparation. According to Lewander¹⁷, rats remain in a withdrawal state for 4 to 5 d after termination of chronic treatment with amphetamine. Similarly, 96 h after cessation of chronic treatment with *d*-amphetamine, specific ^3H -dihydroalprenolol binding to rat brain particulate fraction

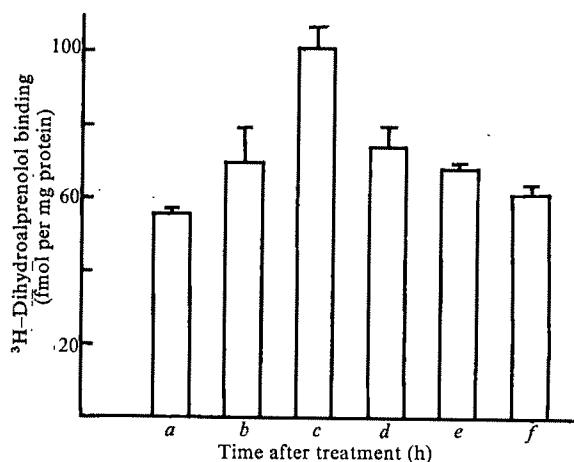


Fig. 1 Effect of chronic administration of *d*-amphetamine on specific binding of ^3H -dihydroalprenolol. The drug was administered intraperitoneally at a dose of 10 mg per kg daily for a period of six weeks. The rats were killed at the indicated times after drug administration by decapitation and the brains were rapidly removed and chilled in ice-cold 0.9% NaCl. The whole brain minus cerebellum was homogenised with a Brinkman Polytron, in 20 volumes of ice-cold 50 mM Tris buffer (pH 8.0 at 25 °C), and centrifuged at 49,000g for 15 min. The pellet was homogenised in the same buffer and centrifuged as before. The pellet was finally resuspended in 50 volumes of 50 mM Tris buffer and used fresh for the binding assay. ^3H -Dihydroalprenolol (32 Ci mmol⁻¹) binding was determined at 25 °C by incubating 0.97 ml of the brain particulate suspension, 2.0 nM ^3H -dihydroalprenolol and sufficient distilled water to make 1 ml as the final volume of the reaction mixture. After 20 min incubation, the reaction mixtures were filtered under reduced pressure through Whatman glass fibre filters (GF/B). The filters were rinsed four times with 4 ml ice-cold Tris buffer to remove most of the unbound radioactive ligand. The filter papers were dried and placed in a counting vial containing 10 ml of scintiverse (Fisher) and counted in a Packard Tri-carb liquid scintillation spectrometer (Model 3380). Corrections were made for nonspecific accumulation of radioactivity by assaying parallel incubations which contained large excess (100 μM) of noradrenaline. Specific binding defined as the difference between total and nonspecific radioactivity was 60-70% of the total radioactive counts. Results shown are averages of nine determinations from three rat brains at each time point, except control where six animals were used. S.E.M. values are indicated by the bars. This experiment has been replicated twice. a, Control; b, 1 h ($P > 0.05$); c, 12 h ($P < 0.001$); d, 24 h ($P < 0.01$); e, 48 h ($P < 0.001$); f, 96 h ($P > 0.05$).

was not significantly different from that obtained with the control preparations (Fig. 1). This would suggest that β -adrenergic receptor supersensitivity may be seen during the withdrawal state following chronic treatment with *d*-amphetamine. Nevertheless, more evidence is needed in support of this possibility.

Both the selective augmentations of motor behaviours in animals and the psychosis in humans associated with chronic amphetamine abuse may reflect a change in catecholaminergic receptor sensitivity. Ellinwood *et al.*¹⁸ proposed that the substantial depletion of catecholamines that accompanies long-term amphetamine treatment allows certain central catecholamine receptors to become supersensitive. This may account for the heightened psychomotor stimulation produced by subsequent injections of amphetamine. The results shown in Fig. 1 are the first biochemical demonstration of the central β -adrenergic receptor supersensitivity after chronic amphetamine intoxication.

Progressive changes in hyperactivity and stereotyped behaviour as a function of the multiple administration of *d*-amphetamine have been considered to be due to the development of reverse tolerance^{19–21}. In guinea pigs²² and in rats²³ readministration of *d*-amphetamine after cessation of daily drug treatment results in a faster onset and also an increase in the intensity of stereotyped behaviour, while administration of apomorphine only affects the faster onset of biological response. Since apomorphine is known to be an agonist of dopamine receptors, these results may suggest that supersensitivity of dopaminergic receptors is related to the decreased latency only and probably does not account for all indices of reversed tolerance. On the other hand, since there is some evidence of involvement of the noradrenergic system in amphetamine induced stereotyped behaviour²⁰, it is suggested that supersensitivity of β -adrenergic receptors may contribute, in part, to the enhancement of the intensity of stereotyped behaviour during chronic amphetamine intoxication.

Thus, chronic treatment with *d*-amphetamine causes a supersensitivity of β -adrenergic receptors in rat brain. This supersensitivity of β -adrenergic receptors may be involved, in part, in the withdrawal reaction to the chronic administration of the drug, tolerance to anorexic effects of amphetamines and enhancement of the intensity of stereotyped behaviour which may lead to psychosis in humans.

This work was supported in part by grants from the US NIH and the AHA, and a postdoctoral fellowship (to V.K.S.) from the Genesee Valley Heart Association.

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Antibodies to thaumatin as a model of the sweet taste receptor

In developing a radioimmunoassay for thaumatin, a sweet-tasting plant protein, we have found that antibodies raised against the molecule cross-react with a wide variety of non-protein sweet substances. The results suggest that some structural feature of thaumatin which interacts with the sweet-taste receptor is also the major antigenic determinant. We suggest that antibodies raised against thaumatin may provide a novel method for measuring 'sweetness' *in vitro* and may throw light on the nature of interactions between sweet substances and taste receptors.

Thaumatin occurs in the aril of fruit of the West African plant *Thaumatococcus danielli* (Bentham). Although the sweet properties were first reported in 1855¹, the active principle was not identified as a protein until recently^{2,3}. There are at least two proteins, designated as thaumatins I and II, which differ

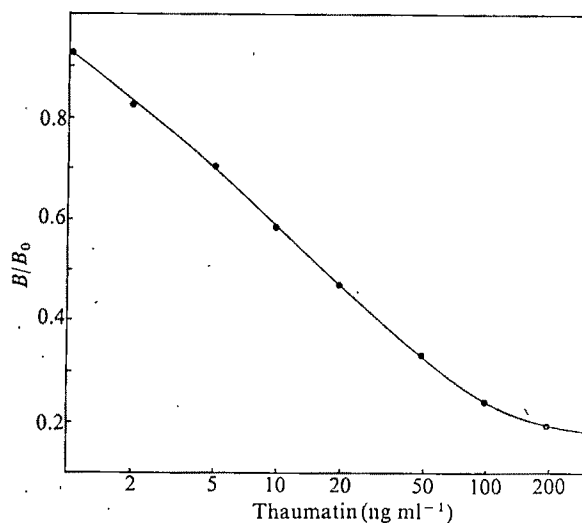


Fig. 1 Standard curve for thaumatin using the coated-tube radioimmunoassay. Thaumatin I, extracted and purified by the method of Higginbotham⁴, was used to raise antisera. Three adult male New Zealand white rabbits each received multiple intradermal injections of a total of 4.0 mg of thaumatin in Freund's complete adjuvant. After a single booster injection at 12 weeks suitable antisera were obtained. Purified thaumatin was labelled with ¹²⁵I in a 1:1 molar ratio using either chloramine-T⁵ or lactoperoxidase⁶, both techniques giving satisfactory incorporation. The labelled protein was separated from unreacted iodine by gel filtration using Sephadex G-50 (fine). A solid phase radioimmunoassay was developed with antibody-coated tubes, using a method previously described⁷ for peptide hormones. Antiserum was diluted in 0.05 M carbonate buffer (pH 9.6), 0.3 ml aliquots were placed in 1-ml plastic tubes for 2 h at 4 °C and the tubes were then aspirated, washed and allowed to dry. An antiserum dilution of 1:50,000 deposited sufficient antibody to bind approximately 50% of 3 pg of ¹²⁵I-labelled thaumatin in 200 μ l of 0.02 M citric acid-phosphate buffer (pH 5.8) containing 0.15 M NaCl and 0.1% bovine serum albumin. The assay was carried out at room temperature and the antibody-bound and free labelled thaumatin were separated by aspiration and washing, after incubation overnight. Increasing concentrations of unlabelled thaumatin displaced the binding of labelled thaumatin, as shown. B, Counts bound to antibody, B₀ = counts bound to antibody in the absence of unlabelled thaumatin. Nonspecific binding to 'uncoated' tubes was 0.03.

only slightly in composition and structure. After purification⁴ their sweetness is approximately 2×10^6 times sweeter than sucrose, when compared on a molar basis.

A solid-phase radioimmunoassay procedure was developed, and Fig. 1 illustrates a typical standard curve. The binding of labelled thaumatin to the antibody coated tubes was readily displaced by the addition of unlabelled thaumatin and the nonspecific binding of labelled thaumatin to tubes without antibody was small. The system thus provides a simple and sensitive procedure for the measurement of thaumatin in biochemical studies of this protein or for quality control where thaumatin is used as a food additive in low, palatable concentrations.

The finding that other non-protein sweet substances cross-reacted with the antisera arose by chance when the assay was used with a soft-drink formulation which contained 5.5×10^{-4} M sodium saccharin in the absence of thaumatin and where significant displacement was observed. A wide variety of sweet substances were tested for their ability to displace labelled thaumatin in the assay, including aspartame (1-aspartyl-L-phenylalanyl methyl ester⁸), calcium cyclamate, neohesperidin dihydrochalcone⁹, 4,1¹,6,6¹-Cl₄-galactosucrose¹⁰, 1¹,6,6¹-Cl₃-sucrose¹⁰ and monellin¹¹, the sweet protein from *Dioscorea-phyllum cumminsii* (Diels). All of these sweet substances caused significant displacement in the thaumatin assay (Table 1) but did not produce similar interference when tested at much higher concentrations (up to 20 mg ml⁻¹) in radioimmunoassays for luteinising hormone and corticotrophin.

Evidence that these substances were binding to antibodies raised against thaumatin was obtained when the sweet protein monellin, which has a different amino acid composition and structure from thaumatin, was labelled with ¹²⁵I and tested for binding with different concentrations of the antisera. A maximum of 35% binding was obtained when tubes coated with a 1:1,000 dilution of the antiserum against thaumatin were used to incubate 0.5 pg of labelled monellin. When the same amount of labelled monellin was incubated with the antiserum in solution and ethanol precipitation or a second antibody used to separate the bound from free labelled peptide, even greater binding (up to 85%) was observed with a 1:1,000 dilution of the antiserum and parallel binding curves were obtained with lower concentrations. Furthermore, when ¹²⁵I-labelled thaumatin was incubated with several of the sweet substances tested, including monellin, gel chromatography revealed no aggregation or other molecular transformation which might inhibit binding of thaumatin to the antibody and give the appearance of competitive inhibition.

The extent to which other sweet substances bind to antibodies raised against thaumatin can be expressed as the molar ratios required to produce equivalent displacement of labelled thaumatin in the assay and these values are shown in Table 1. Similarly, the sweetness of these various compounds relative to thaumatin can be derived from the reported values for their sweetness relative to a 10% solution of sucrose which is a

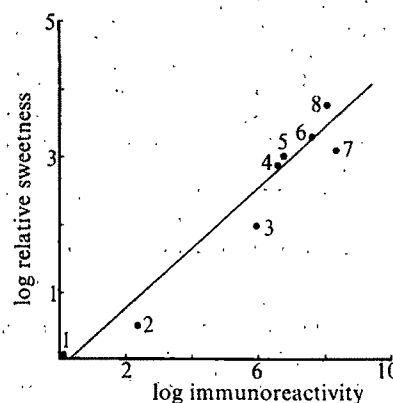


Fig. 2 Regression analysis of the relative sweetness of compounds listed in Table 1 against their immunoreactivity, both plotted on a log scale. Sweetness is expressed as the (log) molar ratio of each substance required to give sweetness equivalent to thaumatin and immunoreactivity as the molar ratio required to give equivalent displacement in the coated tube assay. Key to numbers representing each substance given in Table 1.

conventional standard for such determinations (Table 1). When the immunoreactivity and differential sweetness of these compounds are compared (Fig. 2), the sweeteners are ranked in an almost identical order, with a correlation coefficient value of 0.9606 ($P < 0.001$). It should be noted that sucrose, which is a relatively poor sweetener, will cause displacement of ¹²⁵I-thaumatin from the antibody at high concentrations (50–100 mg ml⁻¹) but at such levels nonspecific effects may influence binding to antibody.

The widely different molecular sizes and chemical structures of the sweet substances which cross-react with antibodies to thaumatin and the excellent correlation between antibody binding and sweet taste suggest that thaumatin has an outstanding structural feature which constitutes the major antigenic determinant and is also the site of interaction with the sweet taste receptor. Thus antibodies raised against this feature will cross-react with other molecules possessing physico-chemical properties which confer sweetness. Interestingly, monellin and aspartame can almost completely displace labelled thaumatin from the antibody at concentrations so low as to almost eliminate the possibility of nonspecific interactions. Although both compounds contain amino acids, the reported sequence of monellin¹² does not suggest that there is a common peptide sequence which is responsible for sweetness.

The remarkable correlation between the recognition of sweet substances by both antibodies and the sweet-taste receptor is probably coincidental but could point to some fundamental relationship in the structure of the recognition site. Binding of sweet substances to antibodies against thaumatin may provide a useful model for the study of interactions at the taste receptor for sweetness and perhaps an *in vitro* procedure for the recognition of novel sweet substances.

Table 1 Comparison of sweet substances

Sweetener	Molecular weight	Relative sweetness		Immunoreactivity
		Relative to 10% sucrose (w/w)	Relative to thaumatin (molar basis)	
(1) Thaumatin	20,000	3,000	1	1
(2) Monellin	10,700	2,000	1/3	2.4×10^3
(3) Neohesperidin dihydrochalcone	642	1,000	1/100	9.0×10^3
(4) 4,1 ¹ ,6,6 ¹ Tetrachlorosucrose*	418	200	1/720	3.0×10^5
(5) L-aspartyl-L-phenylalanyl methyl ester	310	200	1/970	4.0×10^5
(6) 1 ¹ ,6,6 ¹ Trichlorosucrose†	399	100	1/1,500	3.0×10^7
(7) Sodium saccharin	241	150	1/1,600	2.0×10^8
(8) Calcium cyclohexyl sulphamate (cyclamate)	200	50	1/6,000	1.0×10^8

Immunoreactivity is expressed as the molar ratios of each substance required to give equivalent displacement of ¹²⁵I-labelled thaumatin from antibody in the coated tubes assay. Relative sweetness is compared with immunoreactivity in Fig. 2.

* 'Tetrachlorosucrose' is 1¹,6¹-dichloro-1¹,6¹, dideoxy-β-D-fructofuranosyl-4,6 dichloro-4,6 dideoxy-α-D-galactopyranoside.

† 'Trichlorosucrose' is 1¹,6,6¹-trichloro-1¹,6,6¹-trideoxysucrose.

We wonder also whether similar effects could occur with antisera raised against other biologically active substances; for example, could antibodies against the presumed receptor binding site for methionine-enkephalin cross-react with the non-peptide opiate drugs? Such a phenomenon would offer interesting possibilities in screening substances for pharmacological activity.

We thank Mrs Jenny Pennington and Miss Gail Webber for technical assistance, and Professor L. Hough of Queen Elizabeth College, Kensington and Dr R. Khan of Tate & Lyle Group Research and Development for providing samples of the chloro-sucroses.

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Received 4 October; accepted 21 November 1977.

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Phospholipase A inhibition of opiate receptor binding can be reversed by albumin

OPIATE receptors in animal and human brain have been shown to be tightly associated with cell membranes¹⁻³. Our laboratory has reported the solubilisation of an etorphine-receptor complex⁴, but to date it has not been possible to obtain an active receptor in soluble form. Nevertheless, it has been possible to study many biochemical characteristics of the membrane-bound receptor. Its sensitivity to sulphhydryl reagents and a number of other protein reagents^{5,6} as well as to proteolytic enzymes^{1,7} suggests the participation of protein(s) in opiate binding. The evidence is less clear with respect to a function for phospholipids. Opiate binding activity of cell membrane preparations from rat brain has been shown to be exquisitely sensitive to phospholipase A of *Vipera russelli* venom (in the ng ml⁻¹ range)⁸, but very insensitive to phospholipase A present in the venom of *Crotalus adamanteus*¹ or that derived from pig pancreas (Lin & Simon, unpublished). Phospholipase C is inhibitory only in very high concentrations^{1,8}. The great sensitivity of opiate receptors to the Russell's viper enzyme and to ionic and non-ionic detergents^{4,7} points to a possible role for phospholipids in the binding process. We report here that inhibition of opiate binding by phospholipase A can be reversed almost completely by incubation of the enzyme-treated membranes with bovine serum albumin (BSA).

Rat brain membranes were prepared as described previously, with slight modification⁹. Male Sprague-Dawley rats were killed by decapitation. Brains were excised and cerebella were removed. Brains were homogenised in five volumes of 0.05 M Tris-buffer (pH 7.4) with a Brinkmann Polytron, at a power control setting of 5, for two 5-s intervals. The homogenate was centrifuged at 25,000g for 20 min at 4°. The pellet was resuspended in five volumes of 0.32 M sucrose and was stored

at -30° for 2 weeks without any significant loss of opiate binding activity. Frozen homogenate was thawed at room temperature and diluted with 0.05 M Tris buffer (pH 7.4) to give a final dilution of brain tissue of 1 : 60 (w/v).

V. russelli phospholipase A was placed in a boiling water bath for 15 min to destroy any contaminating proteolytic activity. The ability of the phospholipase A to inactivate opiate receptor binding was totally dependent on the presence of calcium⁷. To each reaction mixture CaCl₂ was therefore added at 2 mM concentration. Membranes were incubated with or without phospholipase A (25 ng ml⁻¹) at 37° for 10 min. The reaction was then stopped by chilling the incubate in an ice bath and the enzyme was removed by centrifugation. Phospholipase A-treated and control membranes were resuspended in the same volume of 0.05 M Tris buffer (pH 7.4) with or without 1% BSA. After incubation at 0° for 30 min, the membranes were centrifuged at 27,000g for 10 min, resuspended in the same buffer and centrifuged again. Finally, the pellets were resuspended in the same volume of Tris buffer and stereospecific binding levels of the phospholipase A-treated membranes and their controls were determined as described previously⁸, using an agonist (³H-etorphine), an antagonist (³H-naltrexone) and an endogenous opioid peptide (³H-methionine enkephalin).

The ability of phospholipase A-treated preparations to bind tritiated etorphine, naltrexone or Met-enkephalin was less than 10% that of untreated controls (Table 1). However, the same enzyme-treated membranes after incubation with BSA demonstrated a dramatic recovery of opiate binding activity. As shown in Table 1, binding of all three ligands was now 80-85% of control. Figure 1 shows that fatty acid-poor BSA elicits the same results as non-defatted albumin; other serum albumins, human, porcine, chicken and rabbit, can restore the opiate binding activity of the phospholipase A-treated membranes. Egg albumin was the only albumin tested which did not demonstrate reversal of the effects of phospholipase A. The reason for the lack of restitution of opiate binding by egg albumin is unknown. Of other serum proteins tested, α- and β-globulins demonstrated receptor reactivation of the phospholipase A-treated membranes but γ-globulins were ineffective. We have also incubated trypsin and Pronase-treated membranes with BSA, but restoration of opiate binding activity could not be demonstrated. (Lin & Simon, unpublished). This suggests that the restorative effect of BSA is specific for phospholipase A-treated membranes.

Fatty acids and lysophosphatides, the end products of phospholipolysis have also been shown to be inhibitory to the opiate binding activity of rat brain membranes, but only at relatively high (in the millimolar range) concentrations (Lin & Simon, unpublished). As BSA has been used to remove both

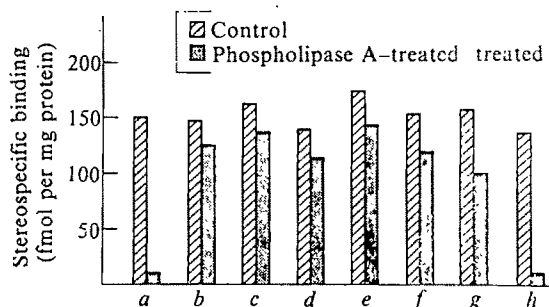


Fig. 1 Restitution of stereospecific opiate binding in phospholipase A-treated rat brain membranes by albumins. Membrane fractions were treated with phospholipase A from *Vipera russelli* venom (11 units per mg protein, Sigma). The procedures of incubation with the albumins are the same as those with BSA described in the text. The determination of stereospecific binding of ³H-etorphine as described in the legend to Table 1. a, no albumin added; b, bovine serum albumin (Pentex, Fraction V); c, bovine fatty acid-poor (Pentex, Fraction V); d, human (Pentex, Fraction V); e, porcine (Sigma, Fraction V); f, chicken (Sigma, Fraction V); g, rabbit (Sigma, Fraction V); h, egg (Sigma, crystallised).

Table 1 Effect of bovine serum albumin on phospholipase A-treated rat brain membranes

Ligand	Phospholipase A	1% BSA	Stereospecific binding (fmol per mg protein)	% of individual control
³ H-etorphine	—	—	136.7	100
	+	—	11.8	8.6
	—	+	126.3	100
	+	+	104.6	82.8
³ H-naltrexone	—	—	139.2	100
	+	—	7.9	5.7
	—	+	134.2	100
	+	+	116.5	86.8
³ H-enkephalin	—	—	61.2	100
	+	—	3.9	6.4
	—	+	78.9	100
	+	+	62.0	78.6

Membrane fractions derived from rat brain were treated with phospholipase A from *Vipera russelli* (11 units per mg protein, Sigma) and incubated with or without BSA (Pentex, Fraction V) as described in the text. Opiate receptor stereospecific binding was assayed by incubation of the phospholipase A-treated and control membranes with 1.1 nM ³H-etorphine (16.5 Ci mmol⁻¹) or 1.8 nM ³H-naltrexone (10.6 Ci mmol⁻¹) with or without 1 μM unlabelled levallorphan at 37° for 15 min. Samples were placed in an ice bath for 10 min before filtration through GF/B filters. ³H-Met-enkephalin (15.9 Ci mmol⁻¹) was used at a concentration of 4.6 nM and incubation was carried out at 4° for 1.5 h with or without 20 μM unlabelled enkephalin.

fatty acids and lysophosphatides from solutions or from membranes¹⁰, the recovery of binding activity following albumin treatment might result from the removal of the end products of phospholipase A hydrolysis. This hypothesis received some support from the observation that the addition of BSA simultaneously with phospholipase A completely prevented the inhibition of opiate receptor activity. However, the inhibition of opiate binding by added lysolecithin or linoleic acid was not restored by treatment with BSA (unpublished results). Nevertheless, an inhibitory effect elicited by the products of phospholipase A hydrolysis cannot be ruled out, because the endogenous fatty acids and lysophosphatides produced within the membrane may have effects on the opiate receptors at much lower concentrations than the exogenous materials, which were added as sonicated suspensions in buffer.

As can be seen in Table 1, the inhibitory effect of phospholipase A on the binding activity of these membranes for tritiated etorphine, naltrexone and Met-enkephalin was similar and their binding was substantially restored by incubation with BSA. These results provide evidence that the binding of agonist, antagonist and endogenous morphine-like material involves the same or similar binding sites.

The remarkable sensitivity of opiate receptors to the phospholipase A of *Vipera russelli* and the reversibility of this inhibition by albumin may provide a useful tool with which to define the role of phospholipids in the stereospecific binding of opiates and endorphins. Moreover, methods for the solubilisation of active opiate receptors may also depend ultimately on the interrelationship of phospholipids and receptor proteins.

We thank Dr P. Elsbach for helpful discussions out of which came the idea to try BSA. This work was supported by grant DA00017 from the National Institute on Drug Abuse. We acknowledge a financial gift from Hoffmann-La Roche, Inc. Nutley, N.J.

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Received 22 September; accepted 5 December 1977.

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Calcium ion-dependent vesicle fusion in the conversion of proalbumin to albumin

PROALBUMIN is the immediate biosynthetic precursor of albumin¹, and has been shown (in the rat) to contain a N-terminal extension (Arg-Gly-Val-Phe-Arg-Arg)^{2,3} which can be cleaved by trypsin to yield albumin. We have recently found an enzyme in rat liver, tentatively identified as cathepsin B (ref. 4), which converts proalbumin to albumin, and which is inhibited 70% by leupeptin (3 μM) and by tosyl-lysyl-chloro-methyl ketone (TLCK, 10 μM). Purified bovine cathepsin B (provided by Dr A. J. Barrett) has also been found to carry out the conversion of proalbumin. Zühlke *et al.*⁵ have discussed the possibility that cathepsin B might catalyse the conversion of proinsulin; and since double basic residues occur at the cleavage points of a number of proproteins, a common enzymatic mechanism might be involved in their conversion for secretion. Our earlier work⁶ suggested that conversion of proalbumin was a very late event in the secretory pathway, which agrees with the suggestion of Ikehara *et al.*⁷ that conversion occurs in Golgi vesicles *in vivo*. Here, we present evidence that conversion takes place in isolated Golgi vesicles *in vitro*, that the enzyme concerned is probably cathepsin B, and that the conversion of proalbumin to albumin involves a Ca²⁺-requiring intracellular vesicle fusion occurring at or beyond a transit step through the Golgi apparatus.

Rats were killed 15 min after intravenous injection with ³H-valine (that is, at a time when labelled albumin would just be emerging from the liver)⁸. Liver Golgi vesicle preparations were made by a modification of the method of Merritt and Morre⁹. The vesicles were found to contain a mixture of radioactive proalbumin and albumin, the proportions of which varied from preparation to preparation and which averaged

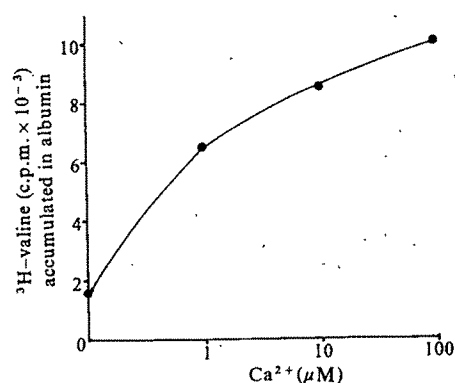


Fig. 1 Calcium requirement for conversion of proalbumin to albumin in Golgi vesicles. Golgi vesicles were separated from the livers of two rats⁹, one of which was given 300 μCi ³H-valine 15 min before death. They were finally suspended in 70 mM sucrose containing 1 mM EGTA. Ca²⁺ was added to give the free Ca²⁺ concentration indicated¹¹. The samples were incubated at pH 6.0 (50 mM 2-[N-morpholino] ethanesulphonic acid) with dithiothreitol 1 mM. Final volume 1 ml. Incubation was for 60 min at 30 °C. The reactions were stopped by addition of 200 μl M Tris-HCl, pH 7.8, 50 μl 10% (w/v) sodium deoxycholate, 750 μg unlabelled rat albumin and 5 ml 150 mM NaCl. Albumin and proalbumin were precipitated with anti-rat albumin and separated using ion-exchange chromatography¹⁷ after release from the precipitate².

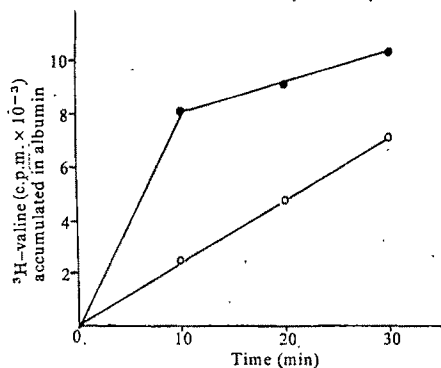


Fig. 2 Effect of a chemical fusogen on conversion of proalbumin in Golgi vesicles. The preparation of Golgi vesicles was as described in Fig. 1, except that just before the final sedimentation, half the vesicles were treated with an equal volume of 80% (w/v) PEG for 1 min at 20 °C. The remainder were treated in the same way, with an equal volume of 70 mM sucrose. They were then chilled, diluted with 70 mM sucrose and sedimented (100,000g, 20 min). They were suspended and incubated as described in Fig. 1. Ca^{2+} was added to give a concentration of 100 μM . The experiment shown is representative of four similar experiments.

about 70% in the form of proalbumin. The yield was approximately 2–3 mg protein per liver. Our Golgi vesicle preparation appeared by electron microscopy to be predominantly small vesicles, with a few cisternal stacks. Marker enzymes showed contamination by endoplasmic reticulum (5%, glucose-6-phosphatase); plasma membranes (6%, 5' nucleotidase) and lysosomes (8%, cathepsin D). No mitochondrial markers were found. The specific activity of UDP-galactose-*N*-acetylglucosamine galactosyl transferase was increased 50-fold over that of the homogenate. In some experiments, the Golgi vesicle fraction (GF_1) of Ehrenreich *et al.*¹⁰ was also tested. The results were identical in the two preparations.

For the experiments, the vesicles were removed from the gradient, sedimented and suspended in 70 mM sucrose containing 1 mM EGTA. The albumin and proalbumin content was determined by disruption of the vesicles with 0.5% (w/v) deoxycholate and immunoprecipitation using anti-albumin. The proteins were separated by chromatography on DEAE cellulose^{2,17}.

A large and reproducible conversion was observed when the vesicles were incubated at pH 6.0 in the presence of Ca^{2+} , the concentrations of which were maintained as described by Portzehl *et al.*¹¹ (Mg^{2+} did not replace Ca^{2+}). Conversion was inhibited 50% by TLCK (10 μM) and 75% by leupeptin (10 μM added partly free and partly entrapped in multilamellar liposomes). Conversion was completely prevented if the vesicles were pretreated with 1 mM DTT and held at pH 7.2 for 30 min at 30 °C before the incubation at pH 6.0. These experiments suggest that cathepsin B or a similar enzyme was responsible for conversion. There was no loss of radioactive protein during conversion and chromatography revealed the presence only of proalbumin and albumin.

It occurred to us that the substrate (proalbumin) and the converting enzyme might be present in separate populations of vesicles which fuse in a controlled manner (see Novikoff's review¹²), an idea which was reinforced by the report that Golgi vesicles can be induced to fuse in the presence of low concentrations of Ca^{2+} (ref. 13). We therefore undertook experiments with polyethylene glycol (PEG) which has been shown to induce fusion in cells and protoplasts^{14–16}. Golgi vesicles were incubated with 40% (w/v) PEG (molecular weight 6,000) for 60 s at 20 °C, and the fusogen removed by centrifugation. There was a marked stimulation in the conversion of proalbumin to albumin in a subsequent incubation at 30 °C, as shown in Fig. 2.

If the PEG is left in contact with the vesicles, there is complete inhibition of conversion. No Ca^{2+} is required during the pretreatment with PEG, but the subsequent conversion has the

same Ca^{2+} requirement as in the absence of PEG treatment (see Fig. 1).

No conversion was seen in smooth endoplasmic reticulum fractions. A crude Golgi cisternae fraction gave only 30% of the conversion seen in Golgi vesicles separated from the same liver. In the experiments, conversion was shown to be intravesicular. Typically, after incubating 60 min in the absence of Ca^{2+} the albumin contained 6,000 c.p.m. ^3H , whereas with Ca^{2+} 100 μM the figure was 11,000. In the supernatant of both albumin was found containing 2,000 c.p.m. Zero-time controls were identical to samples incubated without Ca^{2+} .

These experiments are compatible with the notion that cathepsin B (or a similar enzyme) converts proalbumin to albumin. It is also reasonable to believe that enzyme and substrate are in separate populations of vesicles which fuse in the presence of Ca^{2+} at or near intracellular concentration.

The results suggest that the fused vesicles form a secretory vesicle, which in turn is able to recognise and fuse with the plasma membrane to allow exocytosis. This would account for the fact that proalbumin is not found in extracellular fluids. The results might also explain the action of colchicine which inhibits the conversion of proalbumin to albumin¹⁷ and of parathyroid hormone (pro-PTH) to PTH¹⁸ as well as blocking the secretion of albumin and PTH, and may therefore have a wide interest.

This work was supported by grants from the Cancer Research Campaign, the Vandervell Foundation and the Smith, Kline and French Foundation. We thank Drs R. T. Dean, A. J. Barrett and B. D. Gomperts for assistance.

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Received 3 August; accepted 9 December 1977.

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An unusual interaction between the target of nalidixic acid and novobiocin

BACTERIAL mutation resulting in drug resistance often has pleiotropic effects. Amongst these are alterations in susceptibilities to other drugs. Perhaps the best understood and studied of these interactions are antibiotic resistance mutations that involve genes of ribosomal proteins. The pleiotropic effects induced by ribosomal mutations are usually interpreted by interaction of different ribosomal proteins in the ribosomal organelle. For example, it has been observed that certain ribosomal mutations to neomycin-kanamycin (*nek*) resistance in *Escherichia coli* mask the phenotype of spectinomycin (*spe*) resistance¹. Similarly, there is also interaction between ribosomal ambiguity (*ram*) and the streptomycin (*str*) genes². More recently, it has been observed that there is an interaction of ribosomal (*str*) and RNA polymerase subunit (*rif*) mutations³. I describe here an interaction between mutations involving resistance against two quite different drugs, nalidixic acid and

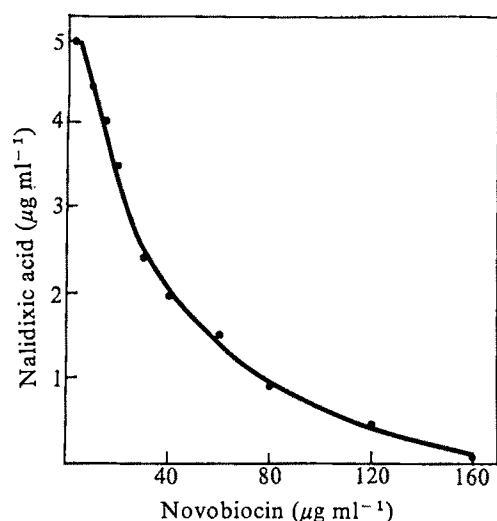


Fig. 1 Effect of drug combination on the growth of *E. coli* KL161. A series of concentrations of novobiocin in the presence of various concentrations of nalidixic acid were prepared in L broth for the drug sensitivity test. The MIC of each drug combination was determined as described in the legend to Table 1. An isobologram was constructed from these values. Each point on the isobologram represents the two drug concentrations that showed growth inhibition when used in combination.

novobiocin, both of which affect DNA replication. This genetic interaction may reflect existence of a protein complex in the DNA replication machinery which has already been postulated on other grounds (see ref. 4).

Nalidixic acid is an extensively used antibacterial drug whose precise mode of action is poorly understood. The primary action of nalidixic acid is inhibition of DNA synthesis⁶. The structurally unrelated drug, novobiocin, has been shown to inhibit DNA synthesis in a manner similar to that of nalidixic acid⁶. The most likely target of novobiocin seems to be DNA gyrase⁷. Despite a similar action of the two drugs, separate targets seem to be involved, as indicated by the distinct map location of nalidixic acid resistance⁸ and novobiocin resistance⁹ in *E. coli*. The fact that both nalidixic acid and novobiocin specifically block DNA replication suggests a possible *in vivo* interaction between the targets of these two drugs. This possibility was examined with a pair of *E. coli* strains KL161 and KL166. The two strains are isogenic except that KL166 is a *nalA* derivative of KL161¹⁰. First, drug sensitivity tests were carried out, and the results of these tests are shown in Table 1. Note that the *nalA* mutation significantly increased novobiocin sensitivity of strain KL166. This uncommon phenomenon is called collateral sensitivity¹¹. The cause of collateral sensitivity is not clear but drug-target interaction is a possibility. For example, the two drug targets may be structurally or functionally dependent on each other. A mutation in one drug target could thus impair the proper function of the other drug target. This point was further pursued with combination drug testing. It is known that drugs acting on different steps of a biochemical pathway frequently show synergism¹². The results presented in Fig. 1 clearly demonstrate a synergistic effect between the two drugs. Furthermore, in rare cases, drug targets may show such

Table 1 Sensitivity of *E. coli* KL161 and KL166 to nalidixic acid and novobiocin

Strain	MIC ($\mu\text{g ml}^{-1}$)	
	Nalidixic acid	Novobiocin
KL161	5	165
KL166	500	70

Drug sensitivity tested by broth dilution in L broth¹³. KL161 and KL166 were grown in L broth at 37 °C to 10 cells per ml. Approximately 10^8 cells from each strain were distributed into a series of tubes containing various concentrations of nalidixic acid or novobiocin. The lowest drug concentration required to arrest cell growth was designated as minimal inhibitory concentration (MIC) of that drug.

Table 2 Frequency of spontaneous mutation to drug resistance *E. coli* KL161 and KL166

Strain	Frequency of mutation to drug resistance			
	Ampicillin	Chloramphenicol	Streptomycin	Novobiocin
KL161	2.3×10^{-9}	1.8×10^{-4}	3.2×10^{-10}	4030×10^{-6}
KL166	6.5×10^{-9}	2.1×10^{-4}	1.4×10^{-10}	7.9×10^{-5}

Mutation frequency is expressed as the ratio of drug resistant colonies to the number of total viable cells. L broth and L agar were used for cell growth and for plating viable cells. L agar containing antibiotics was used for scoring spontaneously occurred drug resistant mutants. The following drug concentrations were used: ampicillin, $10 \mu\text{g ml}^{-1}$; chloramphenicol, $5 \mu\text{g ml}^{-1}$; streptomycin, $100 \mu\text{g ml}^{-1}$; novobiocin, $200 \mu\text{g ml}^{-1}$ (all drugs from Sigma).

a physical interdependence that an alteration of mutation frequency may result¹. For example, a resistance mutation to one drug could alter the frequency of resistance mutation to a second drug. This was found to be the case. The mutation frequency of the two strains was determined with several antibiotics. The results in Table 2 show that KL161 and KL166 differed in their rate of spontaneous mutation to novobiocin resistance by about 500-fold. This is a specific effect, since the frequency of mutations to ampicillin, chloramphenicol and streptomycin resistance were not significantly altered by the *nalA* mutation (Table 2). Results essentially similar to that of novobiocin were also obtained with coumermycin A₁ (data not shown). This similarity is expected since coumermycin A₁ has been shown to share a common target with novobiocin⁷. It should be noted, that the *nalA* phenotype is not masked by novobiocin resistance. Seventy-four doubly resistant mutants were selected by plating KL166 on novobiocin plates. All mutants retained their resistance to nalidixic acid when replicated on nalidixic acid plates.

Novobiocin and coumermycin have been shown to inhibit DNA gyrase activity and DNA replication in *E. coli*⁷. The precise mechanism of action of nalidixic acid is not known. It is possible that the target of nalidixic acid and that of novobiocin act independently and sequentially in DNA replication. Alternatively, the two drug targets may act interdependently in the replication complex. Taken together with published results on the actions of nalidixic acid and novobiocin, the data presented here strongly suggest that the target of nalidixic acid and novobiocin interact *in vivo*. For example, *nalA* mutation may impose a severe conformational strain on DNA gyrase. Thus, only a limited number of mutations in DNA gyrase are viable. This finding is not entirely surprising since a multicomponent complex is probably involved in DNA replication⁴. A mutation in one component could conceivably affect the function of adjacent components.

I thank J. F. Speyer, P. Sawyer and J. D. Karam for helpful suggestions. This work was supported by the General Medical and Faculty Research Appropriation 1977-78.

Note added in proof: The close relationship between the targets of nalidixic acid and novobiocin is also substantiated by two recent reports^{14,15}.

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Received 25 October; accepted 23 November 1977.

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reviews

Anthropology and aerial warfare

J. Z. Young

From Apes to Warlords. The Autobiography (1904–1946) of Solly Zuckerman. By Solly Zuckerman. Pp. 426. (Hamish Hamilton: London; Harper and Row: New York, 1978.) £7.95; \$17.95.

SCIENTISTS to-day probably associate Solly Zuckerman with Whitehall and the London Zoo. He has indeed served science well in both these places, but in this first volume of his autobiography he tells us about two quite different and earlier Sollys. In the first of these earlier incarnations, he laid the foundations of the knowledge that led to the Pill. In the second, he played a part in devising the strategy of the Royal Air Force. Not being either a gynaecologist or historian, I cannot judge how far these memoirs add new facts to what was already known. But it makes compulsive reading to follow how a young South African made his way into the main streams of anthropology, endocrinology and then, so surprisingly, of aerial warfare.

The significance of the work that he did on monkeys is here almost obscured by the diary of meetings with so many different sorts of people. It is difficult now to believe that before Zuckerman's observation of the swollen behind of the female baboon there was no firm knowledge of the nature of the menstrual cycle. That discovery could indeed be regarded as one of the landmarks of the history of human self-knowledge. Of course, it was not made by one man alone; but Solly's description of the baboons was certainly an important factor, although he modestly refers to it, almost in passing, as "one of several clues".

Out of the wide circle of acquaintances that he made in the 1930s came his life with the warlords. It all began from primitive attempts to find out how to protect people against air raids. He and Desmond Bernal organised themselves into an "Oxford Extra-Mural Unit of the Ministry of Home Security". He gives here literally a blow-by-blow account of how they learned about the effects of bombs on people. This section should be as fascinating for scientists and scientific historians as for soldiers and the general public. Beginning with a few experiments with rabbits and guinea pigs, it ended by contributing essential data for the formulation of the strategy



Zuckerman at Oxford in 1935



At Tobruk in 1943

of vast armies and air forces. The method used at all times was a critical testing of hypotheses. The first one to be disproved was the official Medical Research Council view that blast kills by entering the mouth. Disproved by watching a bird fly away as a great gun fired and then showing that the lungs of a rabbit enclosed up to its neck in a box was not damaged by blast. The same combination of observation and experiment was then applied in the study of the results of bombing, first of one room in a lone raid on Banbury and then of hundreds of incidents at Hull and Birmingham. The results of this survey were the basis of one of the disputes about which much has been written between Tizard and Cherwell. Zuckerman claims that it has never been pointed out that the conclusions Cherwell drew from a preliminary version of their report were exactly the opposite of those reached by the authors of the survey. Unlike Cherwell, Bernal and Zuckerman did not believe that area bombing was effective. As Solly movingly concludes, the final proof came in the bombing of Vietnam where "seven million tons brought no victory—only death and destruction."

It is a fascinating story how this knowledge that they obtained came to

make these two scientists influential in the highest quarters. We have excellent glimpses of Mountbatten and Tedder, of Cherwell and Tizard, of Eisenhower and Churchill, and many more, told, it should be said with a simple directness of observation. Of course, he enjoyed meeting all these great men, but on the whole he kept his head. He has a sharp pen and his views are frank and will not always be welcome. He comments about some of the scientific advisors to Harris at Bomber Command that "not all the scientists who had been drawn into Service posts were as questioning and independent in their judgements as they could have been. On occasion, they were constrained by assumptions which uncannily fitted their master's preconceived ideas".

Among scientists, he has never been partial to anthropologists nor they to him. Nor is he much impressed by philosophers. His comment on Freddie Ayer is that he wishes that he had shown "the interest and energy necessary to become familiar with even a small part of science"; and he doubts "whether the growth of the natural sciences has been much influenced over the past 40 or 50 years by the deliberations of academic philosophers."

On the other hand, he sometimes

At the end of the War, 1945

found it hard to believe in the badness of those about him. In the course of the debate over bombing policy, Zuckerman attended one of the mid-night meetings of the Defence Committee. Churchill read out a minute of Cherwell's describing the suggested bombing plan as "the brain child of a biologist who happened to be passing through the Mediterranean". But in spite of this and other evidence of Cherwell's ill-will and bad judgement Solly could never quite bring himself to recognise what an odious man he was, though he notes that Isaiah Berlin

"used to feel uncomfortable in the presence of the Professor. I never did understand why."

So we are introduced to many of the academics, politicians and soldiers who ruled the world in wartime. It is an invigorating story and shows how necessary it still is to persuade people of the value of applying the scientific method to human affairs. We shall wait eagerly for the post-War sequel. □

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Current neuropeptide perspectives

Peptides in Neurobiology. Edited by Harold Gainer. Pp. 464. (Plenum: New York and London, 1977.) \$41.40.

THE principal aim of this book, says the Editor in his preface, is to provide a baseline of information and perspectives in the rapidly moving field of neuropeptides. In this aim I believe it succeeds, but it is never easy reading and it provides, in some of its chapters, a plethora of undigested and indigestible information, too much perhaps for even the most dedicated neurobiologist.

There are 14 chapters, by 23 contributors; and the neuropeptides are described, dissected and delimited in four principal, but discontinuous, subdivisions. In order of appearance these are Biochemical Methodology, Localisation and Analysis, Metabolism and Physiology, Pharmacology and Behaviour.

The opening chapter, by Berta Scharrer, is entitled "Historical Introduction". A great deal more than that, it describes, albeit in less than seven pages, the basic concepts of the modern discipline of neuroendocrinology of which the author, together with her late husband, are the undisputed founders. The ancestral neurone "equally endowed for the dispatch of long distance and strictly localised signals" yields to its successor, the neurosecretory neurone, capable of all degrees of variation between neuronal and glandular function. Acknowledgement of the inherent capacity of neuronal elements for hormone production is qualified by the current view that neurosecretory neurones manufacture chemical mediators to a degree greatly surpassing that of the more "conventional" neurones. Is this really true, or has this function in higher neurones

been overlooked because it has not yet been looked for?

The next four chapters (Stein on fluorecamine as a peptide reagent; Straus and Yalow on radioimmunoassay; Sternberger on immunocytochemistry; Leeman, Mroz and Carraway on the isolation of substance P and neurotensin) comprise the main section on "Methodology". Individually excellent, they are not equally germane to the general argument of the work. Usefully set down here, however, they will not need to be repeated when the neuropeptide story is extended by further instalments. Despite editorial denials, there is some degree of overlap in this section.

Brownstein's chapter (6) which follows, begins with some historical aspects of the localisation of neuropeptides and continues with a presentation of the results of microdissection and microassays, particularly as applied to the hypothalamus. Accompanying tables give the quantified distribution of LHRH, TRH, somatostatin, vasopressin and oxytocin.

Next, Reichelt and Edminson append a long list of oligopeptides found in the CNS, discussing their role and ending with the working hypothesis that they represent "the final common pathway of multisignal integration". This exciting but as yet unverified notion gives way to a chapter by Harold Gainer and his associates on the biosynthesis of neurohypophyseal peptides and neurophysin. The work reported concerns the hypothalamo-hypophyseal system of the rat, concentrating on the time course of events by which precursors and peptides are first synthesised and then transported from soma to axon and axon terminal.

A mainly methodological chapter by Marks deals with the conversion of prohormones by specific and non-specific peptidases, and useful lists of the latter are included. This very important field is both lucidly and comprehensively dealt with by the author.

A virtually complete survey of peptides in invertebrate nervous systems

is given by Nora Frontali (with Harold Gainer). The authors conclude that the relative prominence of neurosecretion in invertebrates reflects a paucity of endocrine systems and the lowly phylogenetic position of the neurone. The physiological role of neuropeptides is discussed by Jeffery Barker, and his chapter provides definitions of neurohormone and neurotransmitter, as well as listing the distinctions between them. This is a subject where complete agreement has yet to be reached, but Barker concludes that neurohormonal peptides can cause changes in neuronal membranes which are quite different from those produced by putative neurotransmitters.

The concluding three chapters are written by Cooke (on electrical activity and control of peptide release), Klee (on endogenous opiate peptides), and De Wied and Gispen (on behavioural effects). The first of these, devoted to work on the crustacean X-organ sinus gland neurosecretory system, provides evidence that all peptidergic neurones have full electrical activity. Klee, giving an up-to-date but concise account of the present status of the enkephalins and endorphins, is nevertheless moved to supply an even more up-to-date addendum. This emphasises, if emphasis is needed, the extraordinary rapid pace of enquiry in this branch of neuropeptidology. De Wied and Gispen end the book with a chapter describing their own well-documented studies in rats on the effects (shuttle box avoidance response, grooming, and so on) of the intracerebral exhibition of vasopressin and oxytocin analogues, ACTH and MSH peptides, and fragments thereof. The relationships described are complex, and not to be comprehended in a single reading.

With few exceptions, as has been stated, the individual chapters composing this book are not easy to read and there is an unavoidable lack of continuity between one chapter and another. A great merit of the book is the speed with which it has been published, a result bought at the expense of a superfluity of misprints, including an amusing inversion (p81) where the famous Italian apparatus is ascribed to Gogli.

Despite these minor criticisms, the book represents a valuable exposition of the present status of peptides in neurobiology, and it will do until it is overtaken by events and by its successor.

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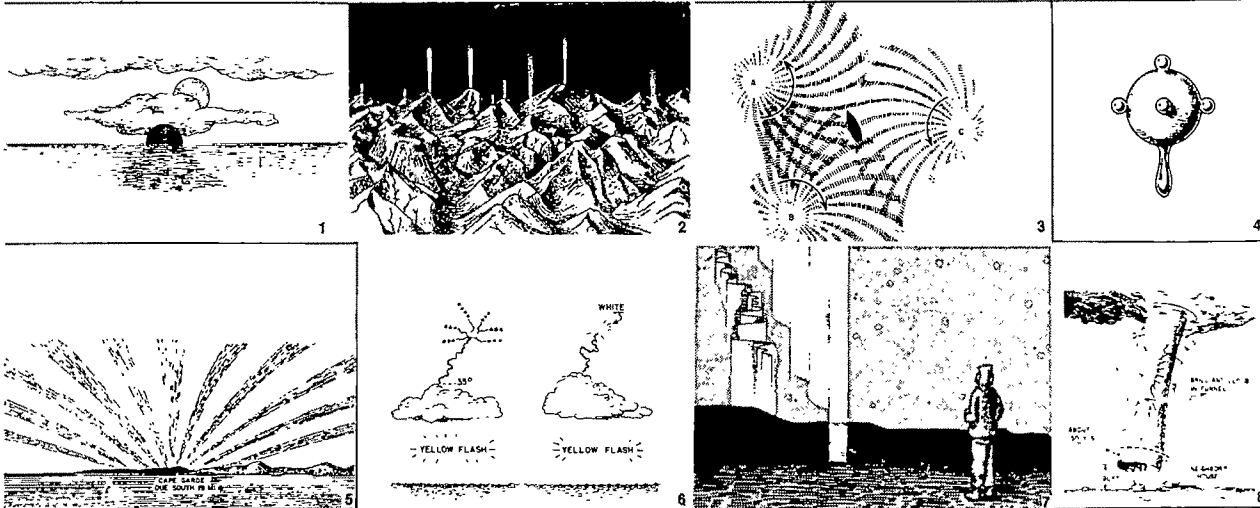
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Experimental Botany: An International Series of Monographs. Volume 13**Plant Response to Wind****J. Grace****December 1977, xli + 204pp., £9.80/\$19.25 0.12.294450.X**

Wind is an important component of the weather as far as crop yield is concerned; for example, it is widely recognised that most crops respond to shelter, but this factor is rarely given sufficient consideration when examining the response of a plant to its environment. This may partly be due to the complexity of equipment required to simulate and examine the effects of wind and also the fact that literature on the subject, until now, has been scattered in journals representing such varied disciplines as botany, forestry, and meteorology. The author brings together this material and discusses shelter effects in terms of the physiology of the plant and the microclimatology of the crop.

Vertebrate Photoreception**edited by H. B. Barlow and P. Fatt****February/March 1978, xvi + 378pp., £14.80/\$29.00 0.12.078950.7**

This volume consists of a collection of papers, the product of a symposium on Photoreception held in London on 2-3 September 1976, under the auspices of the Rank Prize Funds. The subjects covered range from light-induced changes in the visual pigment molecule to the processes operating at synapses in the outer plexiform of the retina, including electrical changes elicited in second-order neurones by activity in photoreceptor cells. Also included is the overall view of visual-signal detection offered by psychophysical studies.

Society for Applied Bacteriology Symposium Series No. 6**Aquatic Microbiology****edited by F. A. Skinner and J. M. Shewan****February/March 1978, xii + 370pp., £15.40/\$30.25 0.12.648030.3**

The field of aquatic microbiology has expanded rapidly in the last decade and the Society for Applied Bacteriology devoted their Summer Conference in 1976 to discussing some of the more important advances which have taken place in this subject. It is intended that this volume, which contains the papers given at the meeting, should provide the practising microbiologist with an up-to-date account of developments in this field. The scope of the book is wide, ranging from the more fundamental aspects of growth and development of microbial communities and their interaction with other organisms, to the practical matters of water purification and supply.

Liquid Metals**An Introduction to the Physics and Chemistry of Metals in the Liquid State****Mitsuo Shimoji****December 1977, xvi + 392pp., £16.50/\$32.35 0.12.641550.1**

This book gives an outline of the present knowledge concerning the properties of liquid metals and alloys. In a liquid metal the conduction electrons are highly mobile, though the order of the ions is lost. In spite of such complicated features considerable progress has been made towards the understanding of liquid metals during the last fifteen years. It is now possible to explain or explore their properties in terms of the quantum theory of electrons in condensed materials and the statistical mechanical theory of classical liquids. Most of the analytical methods used in the book are essentially elementary, and introductory descriptions of the theoretical backgrounds are briefly given.

Development, Function and Evolution of Teeth**edited by P. M. Butler and K. A. Joysey****January 1978, xxii + 524pp., £28.50/\$55.75 0.12.148050.X**

There are perhaps no organs that can be studied in so many different aspects as the teeth. This volume, a synthesis of original research and review articles, is an extensively revised and rearranged version of an important International Symposium on Dental Morphology which was held in Cambridge in 1974. It drew contributions from many disciplines: dental anatomists, anthropologists, embryologists, geneticists, palaeontologists, zoologists and physiologists all gave papers discussing the subject of function, development and evolution of teeth from their own particular viewpoints, and covering the dentition in man and in animals.

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Human and other chromosomes

Molecular Structure of Human Chromosomes: Chromosomes in Biology and Medicine. Edited by J. Y. Yunis. Pp. 336. (Academic: New York, San Francisco and London; 1977.) £17.40; \$24.50.

THIS volume emphasises in respect of human chromosomes the recent advances in technology for the study of the eukaryote nucleus. A collection of nine chapters by different authors, eminent in different specialities of the subject, gives a comprehensive cover, even if with some overlapping.

The first three chapters, by Yunis *et al.*, Macaya *et al.* and Steffensen, respectively, deal with the study of human and other chromosomes at the molecular level. The fourth—by Creagan and Ruddle—is on human gene mapping using new methods, particularly by means of man/rodent somatic cell hybrids. This is by far the best chapter of the book for comprehensiveness, clarity, quality of tabulated information, and soundness of deductions. But what has it to do with the title of the book? A further three chapters, by Bahr, Rao and Dutrillaux, respectively, cover mainly the structure of chromosomes at levels between electron and light microscopy. Finally, chapters 8 and 9, by Pearson and Jones, respectively, deal with the comparative chromosomology of primates—the former on the basis of banding patterns and gene homologies, the latter on the basis of the kinds and distributions of repetitive DNAs.

Processing of heterogeneous nuclear RNA, the bulk of which never gets out of the nucleus; chromosomal localisation of specific DNA sequences; human satellite DNAs; human gene localisation using RNA: DNA *in situ* annealing (I dislike the term “hybridisation”) are all treated extensively and competently in the chapters on the molecular level of chromosome organisation. For readers who are not in this field, the treatment illuminates what has been done and how, and what could be done. It is still too soon to draw even a preliminary model on how chromosome structure relates to function.

Chapter 5, by Bahr on “Chromosomes and Chromatin structure”, deals mainly with the 200-Å chromatin fibres, their folding and unfolding, and their relationship to chromomeres, to sister chromatid exchanges and to re-arrangements. The low magnification electron

micrographs are fascinating. To the interpretations one could apply, for the time being, the Italian saying “se son rose fioriranno” (“if there are roses they will bloom”).

Chapter 6, by Rao on premature chromosome condensation, (PCC), shows how an incidental observation made in the early stages of the study of somatic cell fusion led to interesting developments. PCC occurs in the chromosomes of an interphase cell when it fuses with a mitotic cell. It opens the way to the study, in interphase chromosomes, of the distribution of replicating segments and of some aspects of the induction of chromosome aberrations by radiation.

The introduction by Caspersson and his colleagues in 1970 of the banding techniques into human cytology has fostered a flood of major and minor variations: some add to the resolution of the human chromosomes, and others to the confusion. Chapter 7, by Dutrillaux on “New Chromosome Techniques” contributes more to the latter than to the former, in part at least

on account of poor presentation. It does give, however, a good account on the quenching effect of BUdR incorporation on stainability, and its consequences on producing banding patterns, on making sister chromatid exchanges detectable, and so on.

The final two chapters are stimulating, clear, well written and informative. They discuss the light that banding patterns, distributions of homologous genes and distributions of repetitive DNAs throw on primate evolution.

This book is likely to be useful to outsiders or to people working on one approach to human chromosome structure and function, who wish to know something about other approaches. Abundantly obvious is its lack of unity in style, level and coverage, typical of the present plague of multi-author publications based on editorship without dictatorship.

G. Pontecorvo

G. Pontecorvo was on the research staff of the Imperial Cancer Research Fund, London, until his retirement in 1975.

Liquid crystalline state

Liquid Crystals. By S. Chandrasekhar. Pp. 352. (Cambridge University: Cambridge, London and New York, 1977.) £18.

EVERYONE has now heard of liquid crystals and seen a liquid crystal display device. Few know very much about these materials, even physical scientists, although this situation is now changing quite rapidly. The liquid crystalline state of matter is very common—the fact that the number of known examples is not more than several thousand is probably only because more have not been sought or many have not been recognised. The past decade has seen a great upsurge of work in this area, particularly because of the increasing use of the materials in display devices but also because the time was ripe from a purely scientific point of view.

The use of the name liquid crystal is sometimes criticised and the word ‘Mesophase’ is often preferred. The hybrid name, however, is evocative of the mystery and attraction of the materials, but the mongrel offspring is more than simply a half of each parent. Indeed, both the liquid and the crystal aspects of behaviour are strong, but much of the interest and the usefulness of the materials resides in the essentially hybrid properties.

As is usual in a field of research at this stage of development, a variety of textbooks and monographs have recently appeared and there is necessarily some

overlap among them. Professor Chandrasekhar's monograph is a valuable addition to this still small collection. It inevitably invites comparison on the one hand with the introductory textbook by Priestley, Wojtowicz and Sheng, and on the other hand with the elegant and unique monograph on the *Physics of Liquid Crystals* by de Gennes. They all have their place, in that the coverage, approach and level of treatment are significantly different.

Chandrasekhar's book is essentially an extended critical review, primarily of nematics and cholesterics. After a brief introduction (10 pages) there follows a chapter on ‘Statistical Theories of Nematic Order’ (84 pages) and ‘Continuum Theory of the Nematic State’ (89 pages). There is an extensive discussion of Cholesteric Liquid Crystals (88 pages) and the final chapter on Smectic Liquid Crystals is the shortest (47 pages). This deals only with the smectic A and smectic C phases and does not reflect the extent of current interest and activity in the various smectic phases, but perhaps does reflect the current state of detailed understanding of them.

This is a physicist's book rather than a chemist's, and is certainly not one for beginners. One of the difficulties of working in this area is that many disciplines must be brought to bear on a problem and few scientists can be expert enough in all. In this respect Chandrasekhar's book will be a valuable addition to the libraries of those already actively working on liquid crystals as well as those contemplating the adventure.

A. J. Leadbetter

A. J. Leadbetter is Professor of Physical Chemistry at the University of Exeter, UK.

Basic processes in protein biosynthesis

Molecular Mechanisms of Protein Biosynthesis. Edited by Herbert Weissbach and Sidney Pestka. Pp. 720. (Academic: New York and London, 1977.) £39.05; \$55.

In the mid-1960s, the genetic code had been solved and most of the basic processes in protein biosynthesis defined. During the past ten years, knowledge of the detailed mechanisms has grown enormously by contributions from different disciplines and this is reviewed in the collection of 13 articles edited by Weissbach and Pestka.

As Lipmann remarks in his introduction, the pairing of nucleic acid bases represents the driving force in genetic information transfer. The actual mechanism of protein biosynthesis, however, depends to a larger extent on interactions between nucleic acids and proteins. Of the components involved, the structure of only one, transfer RNA, is known. Consequently, ingenious indirect methods have been used to study molecular interactions in protein biosynthesis and many of the chapters are concerned with these.

The complexity of the ribosome, which in *Escherichia coli* is assembled from 54 different proteins and three RNA molecules, poses formidable problems. These are clearly defined in a stimulating article by Kurland, which serves as an essential introduction for many of the other chapters. The use of cross-linking reagents in determining the relative locations of ribosomal components is also discussed here.

Stöffler and Wittmann describe the ribosomal proteins and studies of their location on the ribosomal surface by electron microscopic visualisation of the attachment of antibodies to individual proteins. This very ingenious method is at present somewhat limited by the resolution of the ribosome in the microscope. The statement that "figuratively, the structure of the 30S subunit resembles an embryo or a telephone receiver" is an attempt to convey an impression of the shape, but it underscores certain ambiguities in resolution. Knowledge of ribosome structure may soon improve, probably from studies of crystalline arrays.

More than 200 genes are involved in the specification of the protein synthetic apparatus, and genetic analysis has played a key role in identifying these components. I. Smith gives an excellent discussion of the genetics and control of biosynthesis of the translational system. Pestka contributes a comprehensive review of protein synthesis inhibitors, principally antibiotics. The use of the latter, in conjunction with

genetics and biochemistry, has been the most powerful method in discriminating individual steps in protein biosynthesis. Even in such a complicated system, affinity labelling has been successful in exploring the functional topology of components, and the critical review by Pellegrini and Cantor will interest anyone using these techniques.

Other chapters discuss transfer RNA and synthetases, messenger RNA and individual steps in protein synthesis. Unfortunately, the volume does not include an account of the *in vitro* assembly of the ribosome, which has played an important role in understanding its structural function.

With all this information, it is remarkable that many of the basic mechanisms in protein synthesis still elude us—notably, the fundamental translocation process by which the mRNA moves relative to the ribosome. The answers to this and other questions

depends on a still more difficult task—study of conformational changes in the ribosome.

In their choice of topics, the editors have skilfully encompassed the field. The chapters overlap to some extent, and cross-referencing would have been useful. Because they are simply designated by numbers, the ribosomal proteins are particularly difficult to follow in discussion, and a tabulation of their properties as an appendix would have greatly helped. In most of the chapters the discussion is set in a good conceptual framework although a few are simply reviews.

The book is a unique presentation of the present situation in this complex field and fulfills an essential need for those involved.

J. D. Smith

J. D. Smith is on the staff of the MRC Laboratory of Molecular Biology, Cambridge, UK.

Classic work of modern physics

Quantum Mechanics of One- and Two-Electron Atoms. By Hans A. Bethe and Edwin E. Salpeter. Pp. 369. (Plenum/Rosetta: New York, 1977.) \$8.95.

THIS work by Bethe and Salpeter first appeared in 1956 in the *Encyclopaedia of Physics*, edited by S. Flügge, and was re-issued in 1957 as a separate book published by Springer. The present paperback edition is changed only by the correction of a few misprints and by the addition of new references where they correct actual errors. The publishers describe it, quite correctly, as "one of the classic works of modern physics", and they are to be congratulated on making it available at a very reasonable price.

The theory of the hydrogen atom, in the Schrödinger and Dirac forms, provides one of the exactly soluble problems of quantum mechanics. Comparison of the theory of radiative corrections with precision measurements (Lamb shift) provides crucial tests of quantum electrodynamics. The theory of the helium atom presents problems which are not exactly soluble; treatment of these problems lays the foundations for the development of the theory of many-electron atoms. The work of Bethe and Salpeter is particularly valuable in the discussion of relativistic effects, which are treated in an unsatisfactory way in many textbooks on atomic structures. The book also contains important sections on atoms in external electric and magnetic fields, and on interactions with radiation.

In their preface to the present edition, the authors say that they have

left the book unchanged not because so little has happened, but because so much has happened. This will, I am sure, be welcomed: it is best that there should be no tampering with classics, even by the authors themselves! The book as it stands is of enduring value.

Yet the reviewer is tempted to ask: what *has* happened in the past 20 years? I would say, first, the development of the theory of continuum states of two-electron systems. Bethe and Salpeter do not exclude discussion of continuum states, in that they give a full discussion of the continuum states of hydrogenic atoms and some discussion of photo-ionisation of two electron atoms. There have, however, been major advances in the theory of electron collisions with one-electron atoms, including studies of elastic and inelastic scattering; doubly-excited states which auto-ionise; and very fundamental problems in the theory of electron-impact ionisation, giving two electrons in the continuum. There are also the problems of radiation by one-electron atoms in external fields due to an added electron, that is to say the theory of spectral line broadening by a perturber electron.

Secondly, there are multi-photon problems. Bethe and Salpeter discuss multi-photon processes such as the $H\ 2s \rightarrow 1s$ decay with emission of two photons but not problems, such as the ionisation of atoms by intense coherent radiation fields, which have become of interest due to developments in laser technology.

Who, I wonder, will write reviews of the later developments with a mastery approaching that of the book by Bethe and Salpeter?

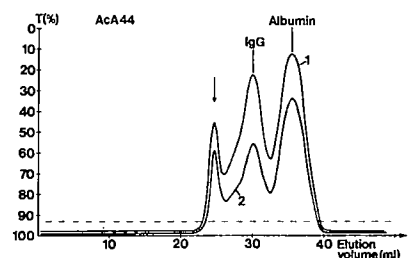
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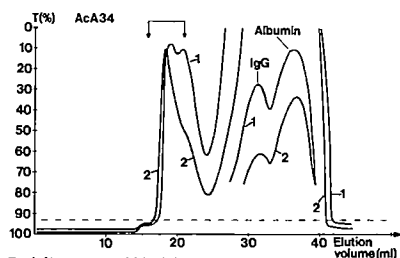
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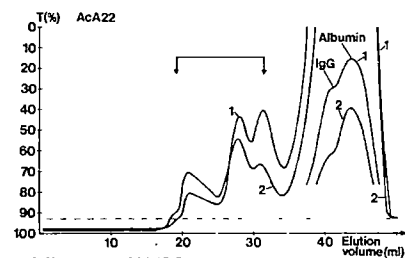
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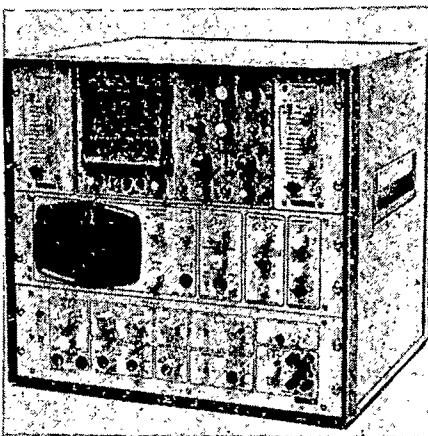
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1131(A)

UNIVERSITY OF BATH BIOCHEMISTRY DEPARTMENT EXPERIMENTAL OFFICER

Applications are invited for the post of Experimental Officer in a team studying the immunochemistry of the acetylcholine receptor protein at the vertebrate neuromuscular junction, financed by the M.R.C. The appointee will have responsibility for establishing and maintaining chicken embryo muscle cells in culture in order to study the acetylcholine receptor on such cells. Experience in tissue culture techniques will be an advantage.

The appointment will be for three years from April 1, 1978.

Commencing salary, £2,955 per annum.

Further particulars and application forms may be obtained from the Personnel Officer, University of Bath, Claverton Down, Bath, quoting reference number 78/14/N. Closing date will be Monday, February 13, 1978.

1154(A)

UNIVERSITY OF NAIROBI KENYA

Applications are invited for the post of PROFESSOR in the Department of Veterinary Anatomy. Applicants should preferably be holders of a first Veterinary of medical professional degree, plus a Ph.D. in one of the following areas: gross Anatomy, Developmental Anatomy, Histology and Embryology. They must also have a considerable teaching and research experience at the University level. The appointee would be required to teach Veterinary and Agriculture under-graduate students, as well as train postgraduate students as well as do research in his/her specific field of specialisation. Familiarity with electron microscopy and a variety of histological techniques would be an advantage. Salary scale: K£4,632 to K£5,562 p.a. (K£1=£1.31 sterling). The British Government may supplement salary by £4,674 p.a. (sterling) for married appointee and £3,384 p.a. (sterling) for single appointee (supplements are reviewed annually and are normally free of all tax) and provide childrens education allowances and holiday visit passages. Terms of service include family passages: superannuation: medical aid scheme: regular overseas leave and various allowances. Detailed applications (2 copies) including curriculum vitae and naming 3 referees should be sent airmail by February 22, 1978 to Registrar (Recruitment and Training) University of Nairobi, P.O. Box 30197, Nairobi, Kenya. Applicants resident in the U.K. should also send one copy to Inter-University Council, 90/91 Tottenham Court Road, London W1P 0DT. Further particulars may be obtained from either address.

1183(A)

UNIVERSITY OF SYDNEY LECTURESHIP/SENIOR LECTURESHIP IN PHYSIOLOGY

The appointee will be required to teach courses to Medical, Science, Pharmacy and Dental students and expected to carry out research in some branch of Physiology, preferably in the area of Gastrointestinal Physiology or Endocrinology. Candidates should have a Ph.D. in Physiology, M.D. or other equivalent higher degree; preference will be given to a candidate with medical qualifications.

The position is permanent but may be filled for three years in the first instance with the possibility of permanency during that time, or in certain cases return fares. (If a suitably qualified candidate with expertise in the indicated research areas is not available a fixed-term contract may be offered to an appropriately experienced Physiologist in another branch of the discipline.)

Salary Range: Lecturers \$A14,851 to \$19,551 p.a. Senior Lecturers \$A19,971 to \$23,299 p.a. Where appropriate a pre-clinical loading will be payable to a medically qualified appointee.

Applications including curriculum vitae, list of publications and names of three referees by February 28, 1978, to Registrar, University of Sydney, NSW 2006, Australia, from whom further information available. Information also available from Association of Commonwealth Universities (Apts), 36 Gordon Square, London WC1H 0PF.

1198(A)

UNIVERSITY OF IDAHO

Assistant or Associate Professor/Ornamental Horticulturist, University of Idaho. Responsibilities: approximately 80% teaching, 20% research. Ph.D. required. Position available after July 1, 1978. Position open until acceptable candidate is selected. Inquiries should be sent to: Ornamental Horticulture Position, Dept. of Plant & Soil Sciences, University of Idaho, Moscow, Idaho 83843. (Ph. 208/885-6276. A.A./E.O. employer and educational institution.

1148(A)

KINGSTON POLYTECHNIC SCHOOL OF CHEMICAL AND PHYSICAL SCIENCES SENIOR TECHNICIAN

to be responsible for the efficient running of an Applied Chemistry laboratory. This is an important laboratory within the School and requires a person with initiative to carry out the large range of duties required using the specialised equipment. Besides a knowledge of chemical engineering equipment, a knowledge of machine-shop practice is necessary as the technician would be responsible for a small mechanical workshop attached to the laboratory. It is also an important part of the post to develop and build equipment necessary to carry out the teaching and research programmes. Duties include maintenance and servicing of equipment plus general duties within the laboratory.

£2,922 to £3,702 plus £312 supplement plus stage 2 supplement plus £285 London allowance.

TECHNICIAN

to be responsible for a postgraduate research laboratory. An interesting post with a wide variety of duties. It is expected that the applicant will have at least 'A' level chemistry and be able to assist both research students and staff in various applications of their research. Other duties include maintenance and servicing of laboratory equipment plus general duties. Day release available.

£1,215 to £2,529 and £312 supplement and stage 2 supplement and £285 London allowance.

Further details and application forms from Assistant Registrar (Personnel), Kingston Polytechnic, Penrhyn Road, Kingston upon Thames KT1 2EE. Tel: 01-549 1366.

1211(A)

Meteorological Office, Bracknell

FLUID DYNAMICIST

for theoretical studies
of the atmosphere

This is an opportunity to join a small team led by Dr Raymond Hide, FRS, investigating the basic principles controlling the motions within rotating fluids.

The person appointed will undertake theoretical studies related to experimental work on the structure, stability and non-linear interactions of various types of wave motions and associated work on atmosphere predictability.

Candidates must be theoretical fluid dynamicists with a 1st or 2nd class honours degree in applied mathematics or theoretical physics. They must also have at least 2 years postgraduate research experience and be able to show evidence of high ability in research. Appointment will be for 3 years as a Junior Research Fellow or Senior Research Fellow, according to experience, with a salary between £3,375 and £5,675.

For further details and an application form (to be returned by 3rd March 1978) write to Civil Service Commission, Alencon Link, Basingstoke, Hants. RG21 1JB, or telephone Basingstoke (0256) 68551 (answering service operates outside office hours). Please quote ref: S/9670.

1166 (A)

MINISTRY OF OVERSEAS
DEVELOPMENT
TROPICAL PRODUCTS INSTITUTE
FRUIT AND VEGETABLE
MARKETING
TECHNOLOGIST

Applications are invited for a scientist to work on marketing technology of fresh fruits and vegetables, including root crops, produced in developing countries. Applicants must be willing to undertake long-term overseas assignments of one year or more, or short-term visits up to four months. Assignments or visits may involve Research & Development work on crop handling and storage and advisory work on market management and design. It is envisaged that this will be a period appointment of three years. The post will be graded Senior Scientific Officer or Higher Scientific Officer with a salary in the range £4,185 to £5,778 for Senior Scientific Officer and £3,254 to £4,454 for Higher Scientific Officer plus £465 Inner London Weighting and Phase 1 & 2 supplements to pay of £522 per annum. Applicants should have a degree in agriculture or a related science and at least five years postgraduate experience for S.S.O. and 2 years for H.S.O. preferably in horticultural crop handling or production. The appointee will work a 41-hour week including meal breaks with 22 days annual leave per year plus 10½ Public and Privilege holidays. The post will be pensionable.

Application forms from Miss C. A. Hall, Tropical Products Institute, 127 Clerkenwell Road, London EC1R 5DB. 1172(A)

UNIVERSITY OF
NOTTINGHAM
DEPARTMENT OF ZOOLOGY
LECTURER IN ZOOLOGY

Applications are invited for the above post. Preference will be given to applicants with research interests in either Immunology or Developmental Biology, although applications from candidates suitably qualified in other branches of Zoology will be considered. The appointment will preferably take place from April 1, 1978, although consideration will be given to applicants unable to start before October 1, 1978. The starting salary will be in the range £3,333 to £3,761 per annum.

Further details and application forms returnable not later than February 13, 1978, are available from the Staff Appointments Officer, University of Nottingham, University Park, Nottingham NG7 2RD. Ref. No. 543. 1160(A)

UNIVERSITY OF
DUNDEE
DEPARTMENT OF
BIOLOGICAL SCIENCES
LECTURESHIP

Applications are invited for a Lectureship in the above department from persons with an interest in any field of Plant Biology other than nitrogen metabolism and photosynthetic prokaryotes.

Salary on the scale £3,333 to £6,655 (currently under review) with initial placing dependent on qualifications and experience. Superannuation under U.S.S. Grant towards removal expenses to Dundee.

Applications (6 copies: overseas applicants 1 copy) quoting Reference EST/17/78J and naming three referees should be lodged by February 23, 1978 with The Secretary, The University, Dundee DD1 4HN, from whom further particulars may be obtained. 1188(A)

Senior Registration Officer (Pharmaceutical)

Our Research Department requires a Senior Registration Officer to register the company's own pharmaceutical products. The person appointed will be responsible for the supervision of a team of officers registering our products and preparing submissions on an international basis. He/she will be expected to contribute to the preparation of drug development programmes and to deputise for the Head of Product Registration when required.

The position will be suitable for a graduate, between 25 and 35 years of age, with 3 to 4 years experience of drug registration and seeking to develop a career in the industry.

The company operates a profit earning bonus scheme, contributory pension scheme and provides help with relocation to the Nottingham area if appropriate.

Please send for application form to: T. W. Flower, Employment Manager (Technical) (U),



The Boots Company Ltd.,
Station Street, Nottingham, NG2 3AA.

1212(A)

UNIVERSITY OF
ABERDEEN
DEPARTMENT OF ZOOLOGY
RESEARCH ASSISTANT

Applications are invited for the post of Research Assistant (M.R.C. supported) to examine the relationship between surface-bound antibody and nutrient absorption in tapeworms. Candidates should have an Honours Degree either in Immunology or in Biochemistry (with experience in immunological techniques).

The appointment will be tenable for three years from a date to be arranged as soon as possible, at an initial salary of £2,904 per annum (plus superannuation benefits).

Further particulars from The Secretary, The University, Aberdeen, with whom applications (2 copies) should be lodged by February 17, 1978. 1142(A)

THE UNIVERSITY
OF LEEDS
DEPARTMENT
OF PHYSIOLOGY

Applications are invited for a LECTURESHIP in Physiology. The Department teaches Science, Medical and Dental students, and has research interests in the major fields of mammalian physiology. Medical qualification valuable but not essential.

Salary on the lecturer scale £3,333 to £6,655.

Applications should be returned to the Registrar, The University, Leeds LS2 9JT, quoting reference number 104/4/D, from whom further details are available. Closing date for applications February 20, 1978. Informal enquiries may be addressed to Professor G. R. Hervey, Department of Physiology. 1168(A)

MEDICAL RESEARCH
COUNCIL

Cellular Immunology Unit
University of Oxford

A vacancy exists for a postdoctoral scientist experienced in

TISSUE CULTURE

The Cellular Immunology Unit is studying functional subsets of lymphocytes and their stem cells and the biochemical nature of their cell-surface molecules. Applicants should be interested in studying the function of cell surface molecules using *in vitro* culture techniques, and also in the production of antibodies by cell fusion.

The initial appointment is likely to be for a 3 year contract on the M.R.C. non-clinical scientific staff with a salary on the appropriate point in the Grade 11 scale (£3,333 to £5,219; under review). Starting date, October 1, 1978 or by agreement.

Applicants should send a curriculum vitae and the names of two referees to Dr A. F. Williams, M.R.C. Cellular Immunology Unit, Sir William Dunn School of Pathology, University of Oxford, Oxford OX1 3RE, U.K. 1137(A)

MERSEYSIDE COUNTY COUNCIL
ASSISTANT KEEPER (PHYSICAL SCIENCES)

£3,395 to £4,214 p.a. including supplements

Applications are invited for the post of Assistant Keeper (Physical Sciences) based at the County Museum, Liverpool. Applicants should possess a degree in an appropriate subject, or the Museums Association Diploma. The successful candidate will have special responsibilities for the operation of the Museum Planetarium and will have particular interests in astronomy. Experience of museum work or work in an associated field is desirable.

Application forms, returnable by February 17, 1978, and further particulars from the Director, Merseyside County Museums, William Brown Street, Liverpool L3 8EN. (Tel: 051-207 0001). 1169(A)

GREATER GLASGOW
HEALTH BOARD—
EASTERN DISTRICT
MEDICAL LABORATORY
TECHNICIAN

Glasgow Royal Infirmary,
Glasgow G4 0SF

A vacancy exists in the University Department of Medicine to co-operate in a team carrying out research on aspects of Thrombosis and Haemostasis. Previous experience is not essential. Applications to the Administrator with names and addresses of two referees. 1189(A)

Biostatistician

The Syntex Research Centre located on the campus of Heriot-Watt University just west of Edinburgh carries out research in pharmacology, toxicology, pharmaceutical development, metabolism and supervision of clinical studies.

SYNTEX
Research Centre

The work is conducted in an innovative and creative atmosphere on a new purpose-built site.

There is now a vacancy for a Biostatistician to provide specialist advice and assistance on the design and subsequent results/analysis of clinical trials and other biological experiments.

Together with an appropriate degree or professional qualification, you will be experienced in the statistical techniques involved in this field, with the ability to relate to other people in a practical, no-nonsense environment. The capability to devise and operate computer analyses would be highly desirable. Age—late 20s upwards.

Salaries and fringe benefits with the company are attractive and relate directly to experience and background.

Please contact: **NICK TEMPLEMAN, PER,**
127 George Street,
Edinburgh 031-225 2736.

PER
Professional
& Executive
Recruitment

Applications from both men and women are welcome

1173(A)

LAKEHEAD UNIVERSITY

Thunder Bay, Ontario, Canada

A position is available for a Post-doctoral Fellow or a Research Associate to investigate the interaction of tetrahydroborate ion with transition metal systems. The position is available immediately and the salary will be in the range \$10,500 to \$13,000 p.a. according to qualifications and experience. Applications, quoting the names of three referees, should be sent to:—

Dr D. G. Holah
Department of Chemistry
Lakehead University
Lakehead University
Canada P7B 5E1.

1147(A)

ROYAL POSTGRADUATE MEDICAL SCHOOL

DEPARTMENT OF MEDICINE
TECHNICIAN

required for Rheumatology Unit as soon as possible until August 1979 to undertake routine and research work involved with Immunological studies of the connective tissue diseases. Minimum qualifications H.N.C. or equivalent, salary up to £4,121 per annum.

Application forms from Personnel Officer, R.P.M.S., 150 Du Cane Road, London W12 quoting Ref. No. 2/360/N. 1135(A)

FIELD STUDIES COUNCIL

WARDEN/DIRECTOR
OF STUDIES

required at Juniper Hall Field Centre, Dorking, Surrey. Good degree in biology or other relevant field science, educ. qualis. desirable. Administrative and field teaching experience essential. Duties include full responsibility for running Centre (staff, finance, academic work etc.) with share of teaching; research opportunities. Present salary scale (under review) £3,480 by £102 by £102 by £99 by £252 by £252 by £246 to £4,533 plus free board and lodging (married accommodation).

Juniper Hall is a gateway to the English landscape, sited in classic field-work region of North Downs and Weald, close to London. Opportunity for person with initiative and new ideas, including more courses for students from Western Europe (present Warden's new post will include overseas recruitment duties). Appointment summer 1978 or sooner, applications must be in by January 30 (weekend interview mid-February), forms and details from Director J. W., Field Studies Council, Preston Montford, Montford Bridge, Shrewsbury SY4 1HW. 1141(A)

INTERNATIONAL LIVESTOCK CENTRE FOR AFRICA

One of the international research institutes funded by the Consultative Group on International Agricultural Research, is expanding information and Training Services and invites applications from qualified and experienced personnel in

- Agriculture Training
- Conference Administration
- Editorial Services
- Library Management
- Publications
- Scientific Translation (English — French — English)

The majority of the staff to be recruited will be based in Addis Ababa, Ethiopia, though some can expect to be based at regional centres in Nairobi, Kenya and Bamako, Mali. Some staff members are required to direct activities in the field of information and training, while others are required to fulfill specific functions within the areas listed. Salaries will vary according to post and experience, but are generally competitive in the range of US \$10,000—32,200 per annum. An outstanding applicant, appointed to a position of wide responsibility, could be offered a salary exceeding US \$32,200 per annum. Most international staff in ILCA are required to be bilingual in English and French.

Most appointments will be made on a two year contract, though applications from those wishing a short term contract (6 or 12 months) are also invited. Applications should be submitted in writing to:

Director
ILCA
P.O. Box 5689
Addis Ababa
Ethiopia

to arrive before 28 February (quoting ref. ITS).

1222 (A)

THE UNIVERSITY OF LEEDS

DEPARTMENT OF
BIOCHEMISTRY

Applications are invited for the post of

LECTURER

in the above Department. The person appointed will participate in teaching biochemistry to science and to medical students. Facilities and opportunities for research are excellent. The appointment will date from October 1, 1978. Salary on the lecturer scale £3,333 to £6,655.

Application forms and further particulars may be obtained from the Registrar, The University, Leeds LS2 9JT, quoting reference number 83/10/D. Closing date for applications March 10, 1978. 1162(A)

HEAD

DEPARTMENT OF GEOLOGY UNIVERSITY OF ILLINOIS at URBANA-CHAMPAIGN

The Department of Geology of the University of Illinois at Urbana-Champaign invites applications for the position of Head of the Department of Geology. Candidates should have the Ph.D. and a demonstrable record of excellence in research and professional leadership in the geological sciences.

The Geology Department currently has 19 full-time faculty members and grants degrees at the Bachelor's, Master's and Doctoral levels. The Head is responsible for maintaining a high standard of quality in both teaching and research within the Department.

Appointment will normally be made at the rank of Full Professor; salary is open. For equal consideration, applications should be received by March 1, 1978. The desired starting date is August 21, 1978. The University of Illinois is an equal opportunity/affirmative action employer. Interested persons should submit a letter of application, vitae and names of references to the Chairperson of the Search Committee:

Professor Charles Wert
Head, Department of Metallurgy
and Mining Eng.
201 Metallurgy and
Mining Building
University of Illinois at
Urbana-Champaign
Urbana, Illinois 61801
(217)333 1440 1080(A)

NIGERIAN INSTITUTE FOR OCEANOGRAPHY AND MARINE RESEARCH, VICTORIA ISLAND, LAGOS VACANCY

POST:—Principal, Marine Fisheries School.

QUALIFICATION:— A good honours degree in Zoology, a post graduate Diploma in Fisheries Management and at least 5 years appropriate field experience.

Salary Grade level 12 to N7,104 by 216 to N7,752.

METHOD OF APPLICATION

Detailed application stating clearly Full Names and Address, Age, Marital Status, Educational and Professional Qualifications obtained with dates, and names of Institutions attended, experience (with positions held, names of Organisations served and Dates), present post and salary, and names and addresses of three personal referees (not relatives), should be forwarded as soon as possible to:—

The Director,
Nigerian Institute for Oceanography
and Marine Research,
P.M.B. 127729,
Victoria Island,
Lagos. 1182(A)

INSTITUTE OF CANCER RESEARCH POSTDOCTORAL SCIENTIST— CYTOGENETICS

required to work at I.C.R., Sutton, Surrey, on a 3 year project designed to evaluate the long-term cytogenetic effects of chemotherapeutic agents on patients with malignant and non-malignant diseases. Previous experience in human cytogenetics essential.

Salary in scale £3,784 to £5,219 p.a. plus London Allowance £450 p.a.

Applications in duplicate with the names of two referees to the Secretary, Institute of Cancer Research, 34 Sumner Place, London, SW7 3NU, quoting ref. 300/G/122. 1130(A)

UNIVERSITY OF
NOTTINGHAM
MEDICAL SCHOOL
PHARMACOLOGY

Applications are invited from those qualified in medicine, pharmacology or cognate disciplines for a LECTURESHIP tenable in the Department of Physiology and Pharmacology (Professor A. T. Birmingham—Professor of Pharmacology).

Pharmacology is taught as one of the basic medical sciences as part of an integrated curriculum for Part I of the Bachelor of Medical Sciences degree. An honours option in Physiology and Pharmacology is available to medical students for Part II of the degree.

There are excellent facilities for research in human and other branches of physiology and pharmacology, including an opportunity to participate in established research programmes. For this appointment an interest in biochemical and pharmacokinetic aspects of pharmacology would be an advantage.

Salary will be within the non-clinical scale for lectures (£3,333 to £6,655 under review) with membership of U.S.S./F.S.S.U. The successful applicant will be expected to take up the appointment as soon as possible.

Further details and application form from the Senior Assistant Registrar, Medical School, Queen's Medical Centre, Nottingham NG7 2UH, to whom completed applications should be returned by February 20, 1978.

1155(A)

THE UNIVERSITY OF
MANCHESTER INSTITUTE
OF SCIENCE AND
TECHNOLOGY

DEPARTMENT OF BUILDING
Research on Energy
Consumption in Buildings

Applications are invited for a Special Research Assistant to work on simulation of building thermal performance and prediction of annual energy consumptions. The work will include the further development, construction and operation of analogue/digital simulation equipment. The successful candidate, male or female, should be familiar either with the thermal behaviour of buildings or with electronic engineering/digital techniques. The work is part of a research contract sponsored by the Building Research Establishment, Watford. Appointment will be for 2 years in the first instance.

The salary will be either £3,761 or £3,975 per annum (Range 1A).

Apply in writing, enclosing a full curriculum vitae including present salary and appointment, to the Registrar, U.M.I.S.T., P.O. Box 88, Manchester M60 1QD, by February 13, 1978. Quote reference B.U.I./10.

1153(A)

THE UNIVERSITY OF
MANCHESTER
ELECTRON MICROSCOPIST
AND MECHANICAL
ENGINEER

There are vacancies for two Research Associates in Geriatric Medicine—an Electron Microscopist (appointment for up to three years) and a Mechanical Engineer (appointment for up to two years). These appointments, preferably postdoctoral, are as part of a new team developing research into the aging bladder and the problems of incontinence. Salary range:—£3,333 to £5,015 p.a. The work will be carried out in the research laboratories of the Department of Geriatric Medicine at the University Hospital of South Manchester, Nell Lane, Manchester M20 8LR, under the overall direction of Professor J. C. Brocklehurst, from whom further details may be obtained.

1196(A)

UNIVERSITY OF SYDNEY
LECTURESHIP IN
VETERINARY CLINICAL
STUDIES

Appointee must have a degree in Veterinary Science acceptable for registration by the Board of Veterinary Surgeons of New South Wales and should have postgraduate experience in horse surgery. Postgraduate qualifications, teaching and research experience an advantage. Appointee will be located primarily in Sydney and be responsible for the conduct of the horse practice in the University Veterinary Hospital and Clinic and will participate in teaching of general and horse surgery.

The position is permanent but may be filled for three years in the first instance with the possibility of permanency during that time, or in certain cases return fares.

Salary Range: \$A14,851 to \$19,551 per annum.

Applications including curriculum vitae, list of publications and names of three referees by February 28, 1978, to the Registrar, University of Sydney, NSW 2006, Australia, from whom further information available. Information also available from Association of Commonwealth Universities (Appts), 36 Gordon Square, London WC1H 0PF.

1199(A)

VERTEBRATE
PALEONTOLOGIST
POSITION

THE UNIVERSITY OF KANSAS has a tenure track joint faculty-curator position for a Vertebrate Paleontologist. The appointment will be 9 months, 1/2-time in the Division of Systematics and Ecology and 12 months, 1/2-time in the Museum of Natural History, at the levels of Assistant Professor and Assistant Curator, respectively. Duties are teaching (undergraduate biology, lower vertebrate paleontology and anatomy courses), curation (in the paleontological collections) and development of an active graduate research program dealing with fossil amphibians and/or reptiles.

Applicant must have Ph.D. by the time duties are assumed or shortly thereafter and be qualified to curate a collection of fossil lower vertebrates. Applicants should send curriculum vitae, a statement of personal goals, and three letters of reference by March 15, 1978, to:

Dr Larry D. Martin
Museum of Natural History
University of Kansas
Lawrence, Kansas 66045

AN EQUAL OPPORTUNITY/
AFFIRMATIVE ACTION
EMPLOYER

1216(A)

LUDWIG INSTITUTE FOR
CANCER RESEARCH
UNIT OF HUMAN CANCER
BIOLOGY

in association with
Royal Marsden Hospital,

Sutton, Surrey

TECHNICIAN

required to undertake responsible work in the cryopreservation of human acute myelogenous leukaemia cells and in examination of proliferative growth in culture. Candidates will be preferred with A level qualifications in biological sciences and with previous experience of tissue culture. Starting salary for this appointment, which will be for 2-3 years' duration, is likely to be in the range of £4,000 to £5,000 p.a. Further particulars may be obtained from the Secretary, London Branch, Ludwig Institute for Cancer Research, c/o The Royal Marsden Hospital, Sutton, Surrey SM2 5PX.

1185(A)



THE FOOD AND AGRICULTURE
ORGANISATION OF THE
UNITED NATIONS

is looking for a

SENIOR TECHNICAL
ADVISOR

FOR A FISHERY, FISH CULTURE AND
AQUACULTURE PROJECT
IN IVORY COAST

The duration of the assignment is three years.

Duties: To direct the activities of a group of experts working on the implementation of a project whose purpose is:

- the creation of a training centre for extension workers;
- the development of river and lake fishery and of fish culture in the East and West Bandama zones;
- pre-promotion of fish culture by the establishment of pilot farms;
- the preparation of studies on the development of fish culture;
- the development of lake and lagoon aquaculture;
- the development of fish culture in rural areas.

Qualifications:

University degree in fishery, fish culture, agronomy, animal husbandry, forestry or any other field of science related to fishery development.

Experience:

Seven to 10 years practical experience in river and lake fishery and/or fish culture.

Languages:

Excellent knowledge of French and good working knowledge of English or Spanish.

Emoluments:

US\$20,000 to 30,000 net (not taxable), plus benefits and cost of living allowances.

Applications, accompanied by detailed curriculum vitae bearing the reference FID 306, should be sent soonest to:

Personnel Officer
Fisheries Department
Food and Agriculture Organisation of the
United Nations
Via delle Terme di Caracalla
00100 Rome
Italy

1139(A)

BUREAU OF HYGIENE AND TROPICAL DISEASES LONDON

I. DIRECTOR

Applications are invited for the post of Director of the Bureau,* which will fall vacant in December 1978. The appointment of the Director-designate will be made in September 1978.

The Bureau's functions are the collection, abstraction and distribution of information from literature published throughout the world on public health, tropical and other diseases, microbiology, food and nutrition etc and, in particular, the production of the monthly publications *Tropical Diseases Bulletin* and *Abstracts on Hygiene*.

The duties of the Director involve responsibility for the efficient management of the Bureau, liaison with Government departments and appropriate organisations to publicize the journals with a view to increasing their circulation world-wide, and the carrying out of the function of chief editor of both publications. Applicants should have medical qualifications registrable in the United Kingdom. Other desirable qualifications could include editorial experience in the medical field, a general knowledge of tropical medicine, medical parasitology and microbiology, an interest in community medicine and epidemiology, and a working knowledge of one or more European languages. Experience and suitability are more important than age, but applicants should preferably be not more than 50 years old.

The salary will be £11,400 and the selected applicant will be required to enter the contributory Universities Superannuation Scheme.

II. ASSISTANT DIRECTOR

Applications are invited for the post of Assistant Director of the Bureau, for which a vacancy may also arise in late 1978.

The duties of the Assistant Director are mainly of an editorial nature. The work could be combined with holding an honorary teaching appointment at the London School of Hygiene and Tropical Medicine which might be offered to a suitable candidate.

Applicants should preferably have medical qualifications registrable in the United Kingdom, but others with qualifications which are comparable to those specified for the Director (see above) are invited to apply. Applicants should preferably be not more than 50 years old.

The commencing salary will be in the range of £8,500 to £10,000 commensurate with the qualifications and experience of the selected applicant, who will be required to enter the contributory Universities Superannuation Scheme.

Applications, accompanied by personal details and the names of two referees, should reach Miss J. M. Dimond, Secretary, BHTD Managing Committee, Eland House, Stag Place, London, SW1E 5DH, not later than 28 February 1978.

*Applicants for the post of Director should state whether they wish to be considered also for the Assistant Director vacancy.

1202(A)

MEMORIAL UNIVERSITY OF NEWFOUNDLAND

Physiologists with a Ph.D. and/or M.D. and postdoctoral training are invited to apply for a faculty position in Physiology, in the Faculty of Medicine, Memorial University of Newfoundland. The position is envisaged to be at the Associate level, although the rank is open to negotiation. The duties include contributing to the teaching of physiology in an integrated medical curriculum as well as some physiology teaching to nursing and science students. The successful candidate should preferably have experience in teaching human and whole animal orientated mammalian physiology and would be expected to have or develop a research program. A strong background in cardiovascular/respiratory and/or renal/body fluids physiology would fit in well with our program. The Faculty of Medicine has recently expanded its facilities into a new Health Science Center which is a part of the Memorial University Campus and will shortly also include the St John's General Hospital. The salary will be commensurate with experience and qualifications and the position is available immediately or by negotiation. Applications, including curriculum vitae and the names and addresses of three references, should be sent to: Dr C. R. Trigg, Chairman, Physiology Search Committee, Faculty of Medicine, Memorial University, St John's, Newfoundland, Canada A1C 5S1. The application deadline is April 15, 1978. 1145(A)

LINCOLN COLLEGE (University College of Agriculture) New Zealand LECTURER IN BIOCHEMISTRY

The Council of Lincoln College invites applications for the position of Lecturer in Biochemistry.

Applicants should have a degree in Science or Agricultural Science, and a postgraduate qualification. Preference will be given to those with experience in Agricultural Biochemistry. Duties will include teaching Biochemistry in the course for Bachelor of Agricultural Science, and the appointee will be expected to carry out research in Biochemistry related to Agriculture.

Commencing salary according to qualifications and experience within the range NZ\$10,239 to \$12,543 per annum. At present salaries are supplemented by an "Interim Special Allowance" of 3.5 per cent.

Travel and removal expenses reimbursed up to specified limits. New Zealand Government Superannuation available.

Conditions of Appointment are obtainable from the Association of Commonwealth Universities, (Appts), 36, Gordon Square, London WC1H 0PF, or from the Registrar of the College.

Applications close on March 6, 1978. 1136(A)

THE UNIVERSITY OF LEEDS DEPARTMENT OF FOOD SCIENCE Opportunities for Postgraduate Research

Applications are invited from good Honours graduates (or those expecting to obtain a good Honours degree within the next academic year) in: Biochemistry; Biological Sciences; Chemistry; Chemical Engineering; Chemical Physics; Mathematics, Physics, or appropriate allied subjects. Applicants should have a desire to apply chemical, biochemical and physical concepts to the solution of problems in food science.

Current departmental interest involves aspects of the chemistry and biochemistry of natural products, the physics of food materials, food microscopy and engineering problems involving food materials.

Enquiries should be addressed to Professor David S. Robinson, Procter Department of Food Science, The University, Leeds LS2 9JT. 1152(A)

WELSH NATIONAL SCHOOL OF MEDICINE (University of Wales) TENOVUS LABORATORIES VELINDRE HOSPITAL WHITCHURCH, CARDIFF RESEARCH OFFICER (BIOCHEMIST)

Applications are invited from suitably qualified persons, preferably post-doctoral, for the post of Research Officer. The appointed person will be involved in the preparation and characterisation of tumour extracts for use in immunological tests concerned with the detection and monitoring of malignant disease. Experience in protein separation desirable. Salary within the range £3,333 to £3,971 (under review); starting point according to qualifications and experience. Application forms (quoting ref. No. 0) may be obtained from the Registrar, Welsh National School of Medicine, Heath Park, Cardiff. Further details obtainable from Dr J. A. V. Pritchard, Velindre Hospital, telephone No. Cardiff 63325 ext. 40. 1165(A)

MUSEUM DIRECTOR THE UNIVERSITY OF TEXAS

at Austin is seeking applicants for the position of Director of the Texas Memorial Museum. The Texas Memorial Museum is a multi-discipline natural history museum. The successful applicant must be qualified in an appropriate academic department.

Salary is commensurate with experience and qualifications of the successful applicant.

Applicants should submit a curriculum vitae, names of 5 references, appropriate statement of interests, and suitable reprints to: Chairman, Museum Search Committee, Burdine Hall 336, The University of Texas at Austin, Austin, Texas 78712.

Application deadline: April 1, 1978. An Equal Opportunity/Affirmative Action Employer. 1220(A)

HELLENIC ANTICANCER INSTITUTE

The Hellenic Anticancer Institute invites applications for a director of the Department of Medical Oncology in the St Savvas Hospital.

The applicants must have proven experience in Medical Oncology and administrative abilities.

Send applications to:
Hellenic Anticancer Institute
171 Alexandras Ave.
Athens, 603

to arrive before March 31, 1978. 1174(A)

M.R.C. CLINICAL RESEARCH CENTRE

(Northwick Park Hospital)

Watford Road, Harrow

Middlesex HA1 3UJ

There is a vacancy for a biochemist with experience in carbohydrate chemistry to join an immunochemistry research team working on structures of the carbohydrate antigens of normal and malignant cells. The programme will involve the purification and the immunochemical and structural analysis of oligosaccharides obtained from glycoprotein antigens. It will offer training in quantitative immunochemistry, and provide special opportunities for research on improved methods of oligosaccharide purification by high pressure liquid chromatography.

The successful applicant, who should be a graduate in science (with Ph.D./D.Phil., or with M.Sc. plus at least 3 additional years' postgraduate experience) will be required to apply for a Medical Research Council training fellowship through the normal annual competition for these awards. The award is tenable for up to 3 years, the closing date for applications is February 10, 1978, and interested persons should send a curriculum vitae to Dr Tenfeizi, from whom further information is available. 1164(A)

UNIVERSITY OF DURHAM LECTURER IN APPLIED PHYSICS

Applications are invited for a Lectureship in the Department of Applied Physics and Electronics tenable from October 1, 1978. Preference will be given to applicants with research interests in applied solid state physics.

Salary will be on the scale £3,333 to £6,655 per annum (under review) plus superannuation.

Applications (3 copies) naming 3 referees should be sent, by February 17, 1978 to the Registrar and Secretary, Science Laboratories, South Road Durham DH1 3LE. 1159(A)

UNIVERSITY COLLEGE OF NORTH WALES, BANGOR RESEARCH ASSISTANT

Salary £2,904 to £3,333 p.a.

Applications are invited for a post of Research Assistant to work in the Xylem Physiology Laboratory of the Department of Forestry and Wood Science, on the control of wood structure within hardwood trees. The project is mainly concerned with environmental effects on the developmental anatomy of wood grown in growth cabinets and in the field. The post is supported by NERC, and is for three years starting April 1, 1978. Applicants should possess a good honours degree in Forestry, Botany, or a closely related subject.

Applications (three copies), giving details of age, qualifications and experience, together with the names and addresses of two referees, should be sent by February 13, 1978, to the Assistant Registrar (Personnel), University College of North Wales, Bangor, Gwynedd LL57 3DG, from whom further particulars may be obtained. 1195(A)

**UNIVERSITY OF
DUNDEE
DEPARTMENT OF
BIOCHEMISTRY
POSTDOCTORAL
BIOCHEMIST**

Biochemists are invited to apply for a Postdoctoral Fellowship to work in collaboration with Dr Brian Burchell on a project grant supported by the Medical Research Council. The aim of the project is to examine the molecular mechanisms involved in the regulation of hepatic microsomal UDP-glucuronyltransferase activity. Applicants should have experience in enzymology and/or lipid chemistry and a knowledge of membrane-bound enzymes.

The appointment will be initially for one year and renewable for a further two years. The position can start immediately, but candidates wishing to begin at any time up to June 1, 1978 will be considered. Starting salary in range 1A of the University Research Staff scales (£3,333 to £3,761, according to age and experience).

Applications, containing a curriculum vitae and the names of two referees, should be sent as soon as possible to The Secretary, The University, Dundee DD1 4HN. Please quote Reference EST/16/78J. 1161(A)

**MEMORIAL UNIVERSITY
OF NEWFOUNDLAND
DEPARTMENT OF GEOLOGY**

The Department of Geology invites applications for three faculty positions in **Sedimentology and Marine Geology** with special reference to the Labrador continental margin (subject to the availability of funds).

One appointment will be a regular faculty position at a salary and rank commensurate with qualifications and experience. The appointee will direct and participate in the activities of a research group working in the following specialities: Mesozoic and Cenozoic clastic sedimentology, clay mineralogy, sedimentary geochemistry and micropaleontology. Applications from suitably qualified candidates with working experience in the petroleum industry will be especially welcome.

Two appointments will be at the rank of Assistant Professor (Research) and will be for a two-year term in the first instance. Applicants should have proven research ability in one (or more) of the specialist fields listed above.

Applications for all three positions, which must include a detailed curriculum vitae and the names and addresses of three referees, should be sent by 14-ch 31, 1978 to: Dr David Skevington, Head, Department of Geology, Memorial University of Newfoundland, St John's, Newfoundland, Canada A1B 3X5. 1170(A)

**UNIVERSITY OF
SOUTHAMPTON
TENOVUS RESEARCH
LABORATORY**

A postdoctoral immunologist or biochemist with some immunological training is required to join a team engaged principally on cell surface studies of leukaemic lymphocytes. Appointment for three years in the first instance. Salary on University Research Fellow Scale 1A, £3,333 to £5,627 p.a. (under review). Enquiries to Professor G. T. Stevenson, Tenovus Research Laboratory, General Hospital, Tremona Road, Southampton SO9 4XY.

Applications to Mr C. W. L. Swann, The University, Southampton SO9 5NH quoting reference 877/RN. 1132(A)

**GREATER GLASGOW
HEALTH BOARD—
WESTERN DISTRICT**

**GRADUATE
SCIENTIST**

**UNIVERSITY DEPARTMENT
OF BACTERIOLOGY
AND IMMUNOLOGY
Western Infirmary, Glasgow**

Applications are invited for the post of Graduate Scientist in the N.H.S., concerned with development and research in Immunology. The appointee is expected to possess a first or upper second class Honours degree in a biological science, preferably microbiology, immunology or biochemistry.

The appointee is expected to participate in research investigations in immunology under the direction of Professor R. G. White. The appointment would be expected to lead to a career in immunology or microbiology within the National Health Service and progress would be encouraged through the examinations of the Royal College of Pathologists in these subjects. Alternatively the appointment could form the basis for investigative work leading to a higher University degree.

Candidates with a Ph.D. and experience in immunology or microbiology could also apply.

Salary will be according to degree and experience since graduation. Applications to: Miss B. S. Young, Sector Administrator, Western Infirmary. 1127(A)

**UNIVERSITY OF READING
DEPARTMENT OF PHYSIOLOGY
AND BIOCHEMISTRY
RESEARCH ASSISTANT**

required as soon as possible for a three-year research project funded by S.R.C. on the neuroendocrine control of ovulation in birds. Applicants should be graduates in physiology, pharmacology or zoology and should have a good background in neurophysiology. An interest in neuroendocrinology would be an advantage.

Starting salary £2,904 p.a. (under review). U.S.S. superannuated.

Apply, quoting Ref. MN. 05A, with the names of two referees to Assistant Bursar (Personnel), University of Reading, Whiteknights, Reading RG6 2AH. 1204(A)

**UNIVERSITY OF GLASGOW
RESEARCH TECHNICIAN**

Applications are invited for the post of Research Technician in the Department of Pathological Biochemistry, Royal Infirmary, Glasgow (Professor H. G. Morgan). The successful candidate, who must have extensive laboratory experience, will assist in the study of the effects of various physiological or pharmacological stimuli on the metabolism of human high density lipoproteins. The post is for one year in the first instance, within the scale of £2,688 to £2,760 p.a. Applications to Dr J. Shepherd, Department of Pathological Biochemistry, Royal Infirmary, Glasgow G4 0SF (041-552-3535, Ext. 5267) within three weeks of appearance of the advertisement. In reply please quote Ref. No. 4047M. 1192(A)

**ZOOLOGISTS
Ecology and Control
of Rodent Pests**

Posts are available in two departments concerned with the control of rodent pests. Each involves field studies of their ecology and behaviour, the development of control methods and the assessment of rodenticide performance. The scientists appointed will also provide training in survey and control methods.

Ministry of Agriculture, Fisheries and Food

Ref: SB/35/AF

The work will include the collection of data to measure the extent of the rodent problem. The post is based at Tolworth with field studies in Powys. Appointment as Higher Scientific Officer.

Ministry of Overseas Development

Ref: SB/1/JA

The appointment will be at Tolworth and the successful candidate will be expected to spend up to 80% of the time overseas. Appointment as Higher Scientific Officer or Senior Scientific Officer.

For both posts candidates must have a good honours degree or equivalent in Zoology/Ecology/Applied Biology and have at least 2 years postgraduate experience, preferably in ecology on control of small wild mammals.

Candidates for appointment as Senior Scientific Officer (£4980—£6575) should normally be aged under 32, for Higher Scientific Officer (£4030—£5250) normally under 30. Starting salary will depend on qualifications and experience.

For further details and application form (to be returned by 17 February 1978) write to Civil Service Commission, Alencon Link, Basingstoke, Hants, RG21 1JB, or telephone Basingstoke (0256) 68551 (answering service operates outside office hours). Please quote appropriate reference. 1129(A)

VICTORIAN COLLEGE OF PHARMACY LTD.

Melbourne Australia

Position of Dean (Principal) of the College

\$30,786 (Australian)

The Council of the College invites applications for the position of Dean of the College from January 1, 1979, when Dean Nigel C. Manning, C.B.E., retires.

The position is that of the chief executive of the College, and the successful applicant will have enjoyed a broad scientific academic background, preferably in the pharmaceutical sciences, and will have extensive academic and administrative experience.

The occupant will receive a professorial salary and an additional allowance of \$4,000 per annum.

Further information on the position may be obtained from the Registrar of the College, with whom applications and the names of three referees should be lodged at 381 Royal Parade, Parkville, Victoria, Australia 3052. The closing date for receipt of applications is April 14, 1978.

Council reserves the right to appoint by invitation.

1146(A)



Wellcome

Senior Technicians

Pharmacology

Opportunities exist in our expanding Pharmacology Department for two senior technicians. The work will be in the general area of biochemical pharmacology and a wide experience in this field will be essential in order to contribute to a variety of projects.

Applicants, who may be male or female, should have an HNC or degree level qualification in an appropriate discipline.

Our Laboratories are situated in pleasant parkland surroundings, within easy reach of Bromley and Beckenham, about 12 miles from Charing Cross. Conditions of employment are attractive and include four weeks' holiday, company pension fund, sick pay scheme, subsidised canteen and generous assistance with relocation expenses where appropriate.

Please apply in writing to
N. J. Michell,
Personnel Officer,
The Wellcome Research Laboratories,
Langley Court, Beckenham,
Kent BR3 3BS,
quoting ref U/841.

1181(A)



THE ROYAL VETERINARY COLLEGE University of London Division of Preclinical Studies DEPARTMENT OF ANATOMY

Applications are invited for the post of
LECTURER IN ANATOMY

The post becomes vacant on September 1, 1978, and is based at Camden Town. The person appointed will be expected to undertake teaching of the anatomy of large animals and will be expected to engage in research.

SALARY SCALE (under review): £3,805 to £7,105 including London Allowance. Initial salary determined by qualifications and experience. Superannuation under the Universities' scheme. Removal expenses up to £650 may be reimbursed in approved circumstances.

Application form and further details from the Assistant Secretary (Personnel), The Royal Veterinary College, Royal College Street, London NW1 0TU. Telephone: 01-387 2898.

CLOSING DATE FOR APPLICATIONS: February 24, 1978. 1125(A)

THE UNIVERSITY OF LEEDS DEPARTMENT OF ANIMAL PHYSIOLOGY AND NUTRITION

Applications are invited for the post of **LECTURER** in the above Department. Applicants must have adequate lecturing experience and should have research interests in the endocrinological aspects of reproduction of farm livestock.

Salary on the lecturer scale £3,333 to £6,655.

Application forms and further particulars may be obtained from the Registrar, The University, Leeds LS2 9JT, quoting reference number 61/2/D. Closing date for applications February 20, 1978.

1151(A)

MASSEY UNIVERSITY Palmerston North, New Zealand **LECTURER IN PLANT PATHOLOGY**

Applications are invited for the above-mentioned position within the Department of Horticulture and Plant Health.

Main responsibilities will be teaching of plant pathology to degree and diploma courses within the Faculty of Agricultural and Horticultural Sciences.

Preference will be given to applicants with doctorate qualifications, some teaching experience, and with proven research interests in one or more of the following fields:— plant virology, plant bacteriology, plant nematology, plant pathogenic fungi.

Commencing salary, according to qualifications and experience, within the range NZ\$10,585 to NZ\$12,969.

Further details of the position and of the University, together with Conditions of Appointment and information to be supplied by applicants, may be obtained from the Association of Commonwealth Universities (Acpus), 36 Gordon Square, London W1P 0PF, or from the Registrar of the University.

Applications close on **February 28, 1978. 1179(A)**

UNIVERSITY OF STRATHCLYDE

Applications are invited for a
LECTURESHP

in the **DEPARTMENT OF APPLIED MICROBIOLOGY**

from persons holding an honours degree and a higher degree, one of which should be in microbiology or a related subject. Candidates should have, preferably, experience in aspects of biotechnology although other interests such as design and use of fermenters, pollution, or biodegradation, will be considered. Relevant industrial experience in lieu of a higher degree would also be acceptable.

Salary scale £3,333 to £6,655 per annum (under review) with placing according to qualifications and experience. Superannuation benefit.

Application forms and further particulars (quoting 2/78) and enclosing a self-addressed envelope (9"x4"), may be obtained from the Academic Appointments Officer, University of Strathclyde, Royal College Building, 204 George Street, Glasgow G1 1XW with whom applications should be lodged by February 11, 1978.

1167(A)

university of wales university college of swansea

Senior Research Assistant

Applications are invited for the vacancy of Senior Research Assistant in the Department of Zoology to work with Dr. N. A. Ratcliffe on an N.E.R.C. sponsored project on invertebrate host defence mechanisms and the influence of environmental parameters on these reactions. Candidates should be near to completing or have completed a Ph.D. in Marine Microbiology, Cell Biology or Immunology and should have experience in the use of radioisotopes and basic microbiological techniques. A knowledge of cell separation and fractionation, ecology and/or histochemistry would also be advantageous.

The appointment, which will date from March 1, 1978, or as soon as possible thereafter, will be for one year in the first instance, with the possibility of renewal for a further two years. The commencing salary will be on a scale up to £3,761 per annum plus U.S.S./U.S.D.P.S. benefits.

Further particulars and application forms (2 copies) may be obtained from the Personnel Officer, University College of Swansea, Singleton Park, Swansea SA2 8PP, to whom they should be returned by **Friday, February 10, 1978. 1124(A)**

RU leiden

UNIVERSITY OF LEIDEN THE NETHERLANDS

FACULTY OF SCIENCE

The DEPARTMENT OF CHEMISTRY invites applications for the tenured position of

Full Professor in Inorganic Chemistry

Applicants should have a broad knowledge and experience in the field of inorganic chemistry. Responsibilities will include teaching. The appointee is expected to coordinate the activities of the inorganic chemistry section.

Applications with curriculum vitae, a list of publications, full details of qualifications and experience (including teaching), references etc. should be addressed within 30 days of this publication to the Secretary of the Department of Chemistry, dr. A. B. Ruigrok, Gorlaeus Laboratories, P.O. Box 9502, Leiden, The Netherlands, mentioning vacancy number 8-010/2407.

1138(A)

UNIVERSITY COLLEGE CARDIFF

Applications are invited for the post of

POSTDOCTORAL

RESEARCH ASSISTANT

in the DEPARTMENT OF BIO-CHEMISTRY to study the nature of the biosynthetic precursors of collagen and elastin. The appointment, which is funded by a grant from the S.R.C. to Dr R. Harwood, is tenable for 3 years and is available as soon as possible. The project will involve the analysis of cell-free translation products and previous experience of nucleic acid biochemistry or protein biosynthesis would be advantageous, though not essential. Salary within Research and Analogous Range IA: £3,761 to £4,190.

Applications (2 copies), together with the names and addresses of two referees, should be forwarded to the Vice-Principal (Administration) and Registrar, University College, P.O. Box 78, Cardiff CF1 1XL, from whom further particulars may be obtained. Closing date **February 27, 1978. Please quote reference 1403. 1187(A)**

**UNIVERSITY OF SYDNEY
LECTURESHIP IN ANIMAL
HUSBANDRY
DEPARTMENT OF
ANIMAL HUSBANDRY**

This position is in the Poultry Husbandry Research Foundation Unit at Camden, New South Wales. Applicants should have extensive postgraduate experience in non-ruminant nutrition, with particular reference to poultry. Practical experience with the poultry and/or pig industries an advantage. He/she will be required to participate in general teaching activities of the Department as well as in his/her own area of special expertise, and to be involved in research and supervision of postgraduate students.

Position is permanent but may be filled for three years in the first instance with the possibility of permanency during that time, or in certain cases return fares.

Salary range: \$A14,851 to \$19,551 per annum.

Applications including curriculum vitae, list of publications and names of three referees by February 28, 1978, to Registrar, University of Sydney, NSW 2006, Australia. Information available from Association of Commonwealth Universities (Appts), 36 Gordon Square, London WC1H 0PF. 1134(A)

**THE UNIVERSITY OF
LIVERPOOL**

Department of Applied Mathematics and Theoretical Physics

Applications are invited for a post of Senior Research Assistant in the Department of Applied Mathematics and Theoretical Physics. The project is financed, by the N.E.R.C., for a period of three years from October 1, 1978. The person appointed will work with Dr P. C. Chatwin on a project entitled "The use of numerical simulation techniques in the analysis of data on the diffusion of chemical and biological species in estuaries." Candidates should have research experience in fluid mechanics (in any appropriate discipline including Applied Mathematics, Oceanography or Engineering), and be prepared to make extensive use of a computer. The post is suitable for persons who expect to have a Ph.D. by the starting date.

The initial salary will be £3,333 per annum on a scale rising to £4, per annum.

Applications, together with the names of two referees, should be received not later than March 6, 1978 by the Registrar, The University, P.O. Box 147, Liverpool L69 3BX, from whom further particulars may be obtained. Quote Ref: RV/653/N. 1140(A)

Senior Research Pharmacologist

A vacancy has arisen at our Welwyn Garden City site for a Senior Research Pharmacologist in the Pharmacology Department.

The Department comprises three sections, cardiovascular, anti-inflammatory and general pharmacology. The successful applicant, male or female, will be required to lead a small project team and contribute to the overall Research effort in the general pharmacology section.

Applications are invited from those with 2 to 3 years' post-doctoral experience who can demonstrate achievements in innovative research.

We offer excellent working conditions in modern and well-equipped laboratories. The salary will be commensurate with experience, additional benefits and facilities include a Christmas Award, contributory pension scheme, free BUPA membership, a subsidised restaurant and, in appropriate cases, assistance towards relocation expenses.

For further information and an application form, please contact the Research Personnel Department (quoting reference RD4) Roche Products Limited, PO Box No 8, Welwyn Garden City, Hertfordshire AL7 3AY, telephone Welwyn Garden 28128. The closing date for applications is 23 February 1978.

1158(A)

ROCHE

**M.R.C. CLINICAL RESEARCH CENTRE
(Northwick Park Hospital)**

Watford Road, Harrow,
Middx. HA1 3UJ

The division of surgical sciences has a vacancy for a postdoctorate to work on immunity to trypanosoma cruzi. This position which is tenable for one year only is suitable for an immunologist, microbiologist, parasitologist or biochemist.

Salary within the range £3,995 to £5,219 p.a. plus £450 p.a. London allowance.

Please contact Dr C. Sanderson on 01-864 5311 ext. 2412 for further details and Joan Tucker-Bull ext. 2685 for application form quoting ref. 128/1/4332. Closing date February 24.

1128(A)

**UNIVERSITY OF
EAST ANGLIA
NORWICH
DIRECTOR OF THE
CLIMATIC RESEARCH
UNIT**

Applications are invited from persons with suitable qualifications and experience for the post of Director of the Climatic Research Unit, which is associated with the School of Environmental Sciences. This position will become vacant on the retirement of the present and founding Director of the Unit, Professor H. H. Lamb. The appointment will be at Professorial or Readership level, according to the age and experience of the successful candidate, and will be tenable from October 1, 1978, or as soon as possible thereafter. Salary will be on the Professorial scale £8,106 to £9,587 or on the Reader scale £6,443 to £7,951 (under review).

Applications (one copy only) giving the names of three persons to whom reference may be made, should be lodged with the Establishment Officer, University of East Anglia, Norwich NR4 7TJ (telephone 0603 56161 ext. 2126) from whom further particulars may be obtained, not later than February 28, 1978. No forms of application are issued. In naming three referees you are particularly requested to give only the names of those who can immediately be approached. 1184(A)

**THE UNIVERSITY OF
LEEDS
DEPARTMENT OF
GENETICS**

Applications are invited for the following posts in the above department:—

1. LECTURESHIP

Applicants from all fields of genetics will be welcome. The post is available from October 1, 1978. Salary on the lecturer scale £3,333 to £6,655 (under review). Application forms and further particulars may be obtained from the Registrar, The University, Leeds LS2 9JT, quoting reference number 50/3/D. Closing date for applications February 16, 1978.

**2. RESEARCH
FELLOWSHIP**

Postdoctoral Research Fellow required for a period up to three years from July 1, 1978, to work in the above department on the biosynthesis and mode of action of cytokinins in the moss *Physcomitrella patens*. Applicants with research experience in either plant physiology or microbial genetics would be especially suitable. Salary on the IA Scale for Research and Analogous Staff: £3,333 to £5,627 (under review). Applications, together with the names of two referees, should be returned to Professor D. J. Cove, Department of Genetics, University of Leeds, Leeds LS2 9JT (from whom further particulars may be obtained), not later than March 14, 1978. 1193(A)

**UNIVERSITY OF
NOTTINGHAM**

DEPARTMENT OF ZOOLOGY

Applications are invited for the post of Post Doctoral Research Associate or Postgraduate Research Assistant. The work involves a study of protein reabsorption in the kidney of a lower vertebrate. Candidates should preferably have an interest in electron microscopy, renal physiology and micro-puncture techniques but previous experience is not essential.

Salary £3,333 per annum.

Application forms returnable not later than February 13, 1978 from the Staff Appointments Officer, University of Nottingham, University Park, Nottingham NG7 2RD. 1197(A)

**UNIVERSITY OF
CAMBRIDGE
DEPARTMENT OF
APPLIED BIOLOGY**

A vacancy exists for a Plant Breeder to continue an existing programme on the development of a high yielding, disease resistant variety of navy bean, *Phaseolus vulgaris*, adapted to the S.E. of England. The project is supported by outside funds for 3 years. Salary in the range £3,119 to £3,975 according to age and qualifications. Further information from and applications to the Secretary, University of Cambridge, Department of Applied Biology, Pembroke Street, Cambridge CB2 3DX by February 9, 1978. 1163(A)

**THE NATIONAL INSTITUTE
FOR HIGHER EDUCATION, LIMERICK
APPLIED MATHEMATICS LECTURER**

The person appointed will have expertise in the application of mathematics to problems in engineering, the physical sciences or business. While an appropriate honours degree is the minimum academic qualification it is likely that the successful applicant will hold a postgraduate qualification. The appointee will contribute to existing courses and to the development of new courses.

SALARY: £5,850 to £7,455 plus £100 p.a. marriage and £70 p.a. child allowances together with other benefits.

Application material available from the Personnel Office, The National Institute for Higher Education, Limerick, to be completed and returned by Friday, February 17, 1978. 1210(A)



FOOD AND AGRICULTURE
ORGANISATION OF THE
UNITED NATIONS

requires a

SENIOR AGRICULTURE ADVISER

for the Latin American Regional Centre for Aquaculture being established in Pirassununga, State of São Paulo, Brazil.

The incumbent will be responsible for the organisation of research teams for implementing systems oriented research on selected production systems and will co-ordinate the overall research and training activities of the centre.

The incumbent should possess:

University degree in biology, zoology, fisheries science or other related disciplines, preferably to the level of Doctor of Philosophy.

At least 10 years' experience in planning and execution of aquaculture research preferably in developing countries.

Applications with detailed Curriculum Vitae, quoting the reference FID 309 should arrive at the following address not later than March 31, 1978.

Personnel Officer
Fisheries Department
Food and Agriculture Organisation
Via delle Terme di Caracalla
00100 Rome, Italy 1190(A)

UNIVERSITY OF SINGAPORE CHEMICAL ENGINEERING

Applications are invited for teaching appointments in the Department of Chemical Engineering. Candidates should possess postgraduate qualifications and have relevant teaching/research/industrial experience. Preference will be given to those who are able to teach in one or more of the following areas: PROCESS CONTROL; THERMODYNAMICS; PLANT DESIGN; FOOD TECHNOLOGY; PETROCHEMICAL PROCESSING. Gross monthly emoluments in the range from S\$1,420 to S\$5,045 approx., the initial amount depending on the candidate's qualifications and experience and the level of appointment offered. In addition, the University pays a 13th month annual allowance of one month's salary in December of each year. Leave, medical, housing and provident fund benefits are also available. Candidates should write to:

The Registrar, University of Singapore, Singapore 10, giving curriculum vitae (bio-data), with full personal particulars and also the names and addresses of three referees. 1175(A)

OXFORD POLYTECHNIC

Technician — Geology

(£2,983 to £3,323 including all supplements)

A technician is required in the Geology section of the Department of Construction. Duties will include special responsibility for cutting thin sections.

Further particulars and application forms may be obtained from The Administrator, Oxford Polytechnic, Oxford OX3 0BP. 1149(A)

UNIVERSITIES OF GLASGOW AND STRATHCLYDE

Applications are invited for a
POSTDOCTORAL
RESEARCH ASSISTANT

in the DEPARTMENT OF PURE AND APPLIED CHEMISTRY funded by S.R.C. for studies of morphologically related ultrasonic relaxation phenomena in polymeric solids to be performed in collaboration with the Groups headed by Professor J. Lamb, Department of Electrical Engineering, University of Glasgow and Professor A. M. North, Department of Pure and Applied Chemistry, University of Strathclyde.

The appointment is for a period of two years.

Salary on the national scale for research and analogous staff Range 1A with a commencing salary within the range £3,333 to £3,761 per annum (under review). Superannuation benefit.

Applications (quoting R2/78) enclosing a curriculum vitae and the names of two referees, should be sent to Dr R. A. Pethrick, Department of Pure and Applied Chemistry, University of Strathclyde, Thomas Graham Building, Glasgow G1 1XL, as soon as possible. Further particulars are available from Dr Pethrick on request. 1186(A)

UNIVERSITY OF QUEENSLAND

Australia

DIRECTOR OF

HERON ISLAND

RESEARCH STATION

The appointee will be responsible for the operation of the Station which provides teaching and research facilities for visiting scientists and students. He or she will be expected to engage in a programme of research and should have experience in conducting research in a field of biology, geology or geography appropriate to a coral reef. A higher degree is desirable.

The appointment will be made to the staff of the University at the level of Reader or Senior Lecturer according to qualifications and experience and will be for a period of three years commencing April 1, 1979.

Salary: Senior Lecturer: \$A19,971 to \$A23,299; Reader: \$A26,365. Other Benefits: Superannuation, travelling and removal expenses. A rent-free house is supplied.

Additional information and application forms are obtainable from the Association of Commonwealth Universities (Apts), 36 Gordon Square, London WC1H 0PF.

Applications close on April 30, 1978. 1180(A)

ACADEMIC GASTROENTEROLOGY POSITIONS AVAILABLE

The State University of New York at Buffalo is establishing a new program in gastroenterology. Positions available at all academic levels; research is an important facet. Laboratory space and initial support funds are available. The program utilizes the clinical facilities of the E. J. Meyer Hospital, a new 750 bed hospital, the Buffalo General Hospital, the Children's Hospital, the Veteran's Administration Hospital and Roswell Park. The basic science department of the University and Roswell Park are available for consultation and collaboration.

Address inquiries and curriculum vitae to: Milton M. Weiser, M.D., Chief, Gastroenterology and Nutrition, SUNY at Buffalo, c/o GI Laboratory, Massachusetts General Hospital, Boston, MA. 02114.

Affirmative Action/Equal Opportunity Employer. 1219(A)

FELLOWSHIPS

UNIVERSITY OF LANCASTER

Department of
Physics

POSTDOCTORAL RESEARCH FELLOW

Applications are invited for a Postdoctoral Research Fellowship funded by the S.R.C. for studies of the breakdown of superfluidity in liquid helium-4 at millikelvin temperatures. The work will be carried out under the direction of Dr P. V. E. McClintock. Applicants should have had considerable experience of low temperature experimental techniques, particularly of measurements below 1 K and preferably including dilution refrigeration; some knowledge of computing would be an advantage.

The appointment, for two years, is on grade 1A with a starting salary in the range (subject to review) £3,333 to £3,975.

Further particulars may be obtained (quoting reference L16/D) from the Establishment Officer, University House, Lancaster LA1 4YW to whom applications (five copies) naming three referees should be sent not later than February 21, 1978. 1194(E)

UNIVERSITY COLLEGE OF NORTH WALES, BANGOR

DEPARTMENT OF
BIOCHEMISTRY AND
SOIL SCIENCE

POSTDOCTORAL RESEARCH FELLOWSHIP PHYTOCHEMISTRY ECOLOGY

Applications are invited for a N.E.R.C. Postdoctoral Fellowship to work in collaboration with Dr R. G. Wyn Jones on the accumulation of organic solutes, particularly nitrogenous compounds in halophytic and xerophytic plants.

Experience in the isolation and characterisation of small molecular weight organic compounds and an interest in plant ecology and taxonomy will be highly desirable. The appointment is for two years and will commence on May 1, 1978, at a salary of £3,333 per annum plus superannuation.

Applications (two copies), together with the names and addresses of two referees should be sent to the Assistant Registrar (Personnel), University College of North Wales, Bangor, Gwynedd LL57 2DG, by February 25, 1978, from whom further details may also be obtained. 1088(E)

QUEEN MARY COLLEGE

University of London
PHYSICS DEPARTMENT

Applications are invited for a POSTDOCTORAL RESEARCH FELLOWSHIP

in Theoretical High Energy Physics for programme on Gauge Field Theory and Quantum Gravity.

Initial salary in range (including London Allowance) £3,805 to £4,233 p.a. (under review).

Further details available from Dr J. M. Charap. Applications, including curriculum vitae, list of publications and names of 2 referees, to be sent to The Registrar (N), Queen Mary College, Mile End Road, London E1 4NS, as soon as possible. 1215(E)

RESEARCH FELLOWSHIP

A POSTDOCTORAL FELLOW required as soon as possible for an appointment of two years with possible extension to three years.

The work will be on the mechanisms by which viruses convert cells from a normal to malignant state, with emphasis on leukaemia-inducing viruses.

Salary with entry according to qualifications and experience within range £4,649 to £5,669.

Further information from Dr J. Wyke (tel: 242 0200, ext. 267/270).

Applications with curriculum vitae and names of two referees, should be sent to:

The Secretary,
Imperial Cancer Research Fund,
Lincoln's Inn Fields,
London WC2A 3PX,

by March 17, 1978, quoting Ref. 61/78. 1209(E)

UNIVERSITY OF CAMBRIDGE BROADBANK FELLOWSHIPS

Applications invited for Fellowships for full time research normally at Cambridge in Biochemistry or Biophysics with special reference to the principles and practice of food preservation. Tenure normally from October 1, 1978, for one year first instance but may be extended for one or two further years. Candidature open, but preference to postdoctoral applicants.

Pensionable salary for postdoctoral Fellow in range £3,150 to £4,000 according to experience and qualifications. Compulsory superannuation.

Applicants should ascertain that there is a laboratory in Cambridge willing to accommodate them and must outline research proposed indicating bearing on principles and practice of food preservation and include full curriculum vitae, copies of papers published or references thereto and names and addresses of at least two referees.

Full details available from and applications (ten copies) to reach the Secretary to the Managers of the Broadbank Fund, Department of Applied Biology, Pembroke Street, Cambridge CB2 3DX, not later than March 1, 1978. 1205(E)

RESEARCH FELLOWSHIP IMPERIAL CANCER RESEARCH FUND LABORATORIES Mill Hill, NW7

A postdoctoral Fellow is required to join a group working on the genetic and biochemical analysis of certain heat activated loci in *Drosophila melanogaster*, using cloned DNA fragments to investigate their organisation and expression.

Appointment will be for two years in the first instance with possible extension for a third year. Salary with entry according to qualifications and experience within range £4,649 to £5,669.

Further information from Dr D. Ish-Horowicz (01-959 3236).

Applications with curriculum vitae and names of two referees, should be sent to The Secretary, Imperial Cancer Research Fund, Lincoln's Inn Fields, London WC2A 3PX, by February 28, 1978. 1208(E)

ONTARIO CANCER INSTITUTE POSTDOCTORAL FELLOWSHIP

available to join a group working on the genetics and molecular biology of murine oncornaviruses and erythropoiesis. Candidates should be about to graduate in virology, biochemistry or cell biology in the spring or summer of 1978. Applicants should send curriculum vitae with three letters of references to Drs T. W. Mak or A. Bernstein, Division of Biological Research, Ontario Cancer Institute, 500 Sherbourne St., Toronto, Ontario, Canada M4X 1K9. Stipend will be \$11,600 to \$12,700 annually. 1093(E)

STUDENTSHIPS

BRITISH MUSEUM (NATURAL HISTORY)

POSTGRADUATE RESEARCH STUDENTSHIPS 1978

Two studentships will be available, tenable for 3 years, at the British Museum (Natural History) and commencing October 1978.

Arrangements will be made with an appropriate university for higher degree registration and joint supervision of the research project.

The research areas for which applications are invited are as follows:-

DEPARTMENT OF BOTANY

1. Evolutionary relationships in the Celastraceae with special reference to *Maytenus*, a source of important anticancer drugs.

DEPARTMENT OF PALAEONTOLOGY

2. Ultrastructure of some fossil Rhodophyta (red algae), and their relationships.

Facilities are available for scanning and transmission electron microscopy, serology, biochemistry and biometrics. In addition, students will have access to the Museum's extensive library.

ELIGIBILITY

Applicants to be aged 27 or less, ordinarily resident in Great Britain, and to have a first or upper-second class honours degree; students graduating in 1978 can be offered awards subject to their degree results reaching this level.

Students interested in applying should write to The Secretary (Postgraduate Studentships), British Museum (Natural History), Cromwell Road, London SW7 5BD, for application forms and conditions of the awards.

Closing date for receipt of completed application forms—March 20, 1978. 1157(F)

THE ANIMAL VIRUS RESEARCH INSTITUTE Pirbright, Woking, Surrey A.R.C. RESEARCH STUDENTSHIPS 1978

Applications are invited from Science students graduating this year for an Agricultural Research Council Research Studentship tenable for up to 3 years at the Animal Virus Research Institute. The award is in the Biochemistry Department, where the successful candidate would be concerned with the characterisation of polypeptides of enteroviruses belonging to the Coxsackie B5 and swine vesicular disease groups. The project is aimed at establishing the regions in the nucleic acid coding for the particular differences in the functional coat proteins.

Details of the awards are available from the Secretary, Animal Virus Research Institute, Pirbright, Woking, Surrey, GU24 0NF. Applications should be received by March 31, 1978. 1217(F)

EXETER COLLEGE OXFORD

The College proposes to elect to an Usher Cunningham Studentship for graduate work in Oxford in any branch of Medical Science.

The Studentship will be tenable from October 1, 1978. The financial and other provisions are at the direction of the College but are likely to be similar to those governing awards made by a Research Council.

Applicants must be under twenty-five years of age and must be or become resident members of Exeter College. They must also be accepted as graduate students by a Faculty Board.

Application with the names of two referees should be made to the Rector by February 25, 1978. 1143(F)

COURSES

British Council Course ADVANCE ELECTRON MICROSCOPY FOR BIOLOGISTS

September 17-29, 1978

in
Norwich

The Director of Studies will be Professor R. Markham, F.R.S., Director of the John Innes Institute.

Recent advances in electron microscopy techniques are of considerable importance in the study of plant tissues, cells, organelles, viruses, macromolecules, nucleic acids and other biological materials. This course aims to provide its participants with experience in these techniques and in the experimental methods used in the preparation of biological specimens for high resolution electron microscopy, the operation of the microscopes and the analysis and interpretation of electron micrographs.

The course, which is residential, is designed for non-UK citizens who are practising electron microscopists who wish to extend their skills in high resolution electron microscopy of biological materials.

Information and forms for registration may be obtained from local Representatives of the British Council or from Courses Department, The British Council, 65 Davies Street, London W1Y 2AA.

Completed application forms must be received in London by May 1, 1978. Fee £475. 1171(D)

UNIVERSITY OF SALFORD DEPARTMENT OF BIOLOGY

Applications are invited for the one year course for the degree of M.Sc. in ENVIRONMENTAL RESOURCES.

Applicants should possess an honours degree in a Biological Science, Geography, or Geology. Graduates in Geography, or Geology will be required to have a sufficient knowledge of Biology to undertake the course.

Further details can be obtained on application to the Course Tutor, Department of Biology, University of Salford, Salford M5 4WT. 1150(D)

INSTITUTE OF PSYCHIATRY, NEUROLOGY AND OPHTHALMOLOGY (British Postgraduate Medical Federation, University of London) POSTGRADUATE COURSE IN NEUROCHEMISTRY

This is a ONE-YEAR Course leading to the degree of M.Sc. in Neurochemistry. It offers graduates in science and medicine training in basic and applied neurochemistry. The Course consists of lectures, seminars and laboratory work and, in the second and third terms, research on a neurochemical topic.

Applicants should have an honours degree in Biochemistry or a Biological subject with some Biochemical component, or be Medically qualified. The Course is recognised by the Medical Research Council as an Advanced Course for the award of Training Scholarships to suitably qualified applicants.

Application for the Academic year October 1978-July 1979 should be addressed to the Secretary, Course in Neurochemistry, Department of Biochemistry, Institute of Psychiatry, De Crespigny Park, Denmark Hill, LONDON SE5 8AF, England. 1200(D)

SYMPOSIUM

PERPIGNAN UNIVERSITY

"Contribution multidisciplinaire à la Systématique: Umbelliferae" 18-21 Mai 1977 (2nd International Symposium on the Umbelliferae) Perpignan, France.

50 Contributions (in English, French, German and Spanish) devoted to taxonomy, biology, evolution, ecology, biogeography, ethnobotany, pharmacology and to agricultural aspects of the Umbelliferae.

Size: 32 by 30, 832 p. plus Indexes, paper bound (offset printing), 200 F.F., A. M. CAUWET & J. CARBONNIER Ed., Perpignan University—66025—Cedex (France). 1201(M)

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Graduate Teaching Assistantships are available for candidates with high academic records who wish to pursue further studies towards the M.Sc., and Ph.D. degrees in chemistry. Excellent research facilities are available in all areas of modern chemical science, ranging from bio-inorganic chemistry to chemical physics. The minimum stipends including summer research assistantship will be \$6,120.00 per annum for first year and \$6,500.00 for students entering with a Master's Degree. The stipends are currently under review. In addition, many special merit awards are made to those Graduate Teaching Assistants who show excellence in the performance of their duties. Numerous graduate scholarships are also available for students with outstanding academic records.

Application forms and further particulars may be obtained from the undersigned:

Professor C. A. McDowell,
Department of Chemistry,
The University of
British Columbia,
Canada V6T 1W5

773(P)

UNIVERSITY OF CAMBRIDGE

Applications are invited from Chemists, Physicists or Geologists for a two-year

POSTDOCTORAL ASSISTANTSHIP

with a small group investigating light element (carbon and nitrogen) geochemistry of lunar samples and meteorites. Practical experience in computerised mass spectrometry and an ability to work with small samples is desirable. Some previous involvement in stable isotope analysis could be advantageous since the group is currently developing techniques in this field which could have considerable application in a number of important research areas.

The salary range is £3,333 to £3,547. Further details from, or curriculum vitae and the names of two referees to, Dr C. T. Pillinger, Department of Mineralogy and Petrology, University of Cambridge, Downing Place, Cambridge CB2 3EW. Telephone No. 0223-64131, ext. 283. 1221(P)

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Enquiries to the Administrator, Sir William Dunn School of Pathology, South Parks Road, Oxford. Tel: Oxford 57321. 1203(L)

CONFERENCES

UNIVERSITY OF READING

A one-day meeting on 'Aspects of Xylem Differentiation' will be held on March 30 in the Department of Botany, University of Reading. Speakers will include Professor P. Gahan, Dr P. Denne, Dr R. Johnson, Dr R. Phillips, Dr J. R. Barnett. Chairman will be Professor R. D. Preston F.R.S. Further details and registration forms from Dr J. Barnett, Plant Science Labs., University of Reading, Whiteknights, Reading RG6 2AS. 1177(C)

AWARDS

LADY TATA MEMORIAL TRUST INTERNATIONAL AWARDS for Research on Leukaemia and Allied Conditions

Academic Year 1978-1979

The Trustees of the Lady Tata Memorial Trust invite applications for Awards (both predoctoral and postdoctoral) for research on leukaemia, in the Academic Year beginning October 1, 1978. In view of the affinity between leukaemia and other forms of neoplastic disease, candidates with programmes of research on any aspect of malignant disease which may throw light on problems of leukaemia will be eligible for consideration. The Trustees specially wish to encourage studies of the leukaemogenic viruses in animals, the epidemiology, natural history, and immunology of leukaemia.

Awards are open to suitably qualified investigators of any nationality, working either in their own institutions or in other centres abroad.

Awards are offered at rates appropriate to the age, seniority, and experience of the candidates, and to the stipends paid at the Institution at which the work will be done, with provision for annual increments, bench expenses and superannuation if required.

Awards are tenable for 1 year in the first instance, renewable up to a maximum of 3 years.

Further particulars and forms of application may be obtained by writing to the Secretary of the (European) Scientific Advisory Committee, Lady Tata Memorial Trust, Chester Beatty Research Institute, Fulham Road, London SW3. Applications must be submitted before March 31, 1978, and awards will be announced by the Trustees in June. 1207(N)

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received by

3.00pm

Friday will

appear in

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nature

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Dr O. Schnepp	(University of Southern California, Los Angeles)
Professor G. Sznatzke	(Ruhr-Universität Bochum)
Dr I. Tinoco, Jr	(University of California, Berkeley)
Dr O. E. Weigang, Jr	(University of Maine)
Professor H. Wynberg	(University of Groningen)

Contributed papers:

A limited number of contributed papers and poster contributions will be included and abstracts of 200 words are invited for consideration.

Registration and Accommodation

No registration fee for membership of the Institute is required and a limited number of grants are available for residents of the N.A.T.O. countries to cover part of the travelling and maintenance expenses involved by membership. Application forms for membership and further information are available from the Director of the Institute, Professor S. F. Mason, Chemistry Department, King's College, Strand, London WC2R 2LS, England. 1176(C)

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Further details from the Organising Secretary, WTE, BHRA Fluid Engineering, Cranfield, Bedford, MK43 0AJ, England.

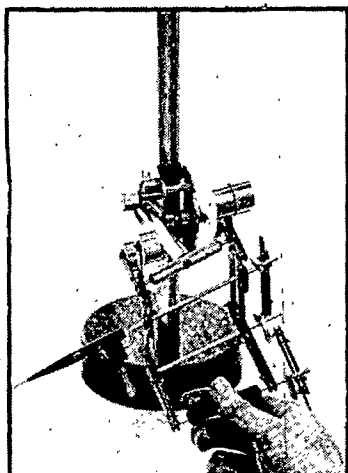
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APPLICATIONS FOR GRANTS MULTIPLE SCLEROSIS RESEARCH

Applications are invited for Grants funded by the National Multiple Sclerosis Society of Australia for research related to Multiple Sclerosis.

Support will be provided for approved clinical or epidemiological work as well as for laboratory-based studies within Australia.

Applications from suitably qualified persons will also be considered for Post-Doctoral Fellowships.

Intending applicants for project grants should write for further information to:

Mr. S. J. Resch,
Chairman,
National Multiple
Sclerosis Society of Australia,
616 Riversdale Road,
Camberwell, 3124, Australia.

Applications close on Friday, March 31, 1978.



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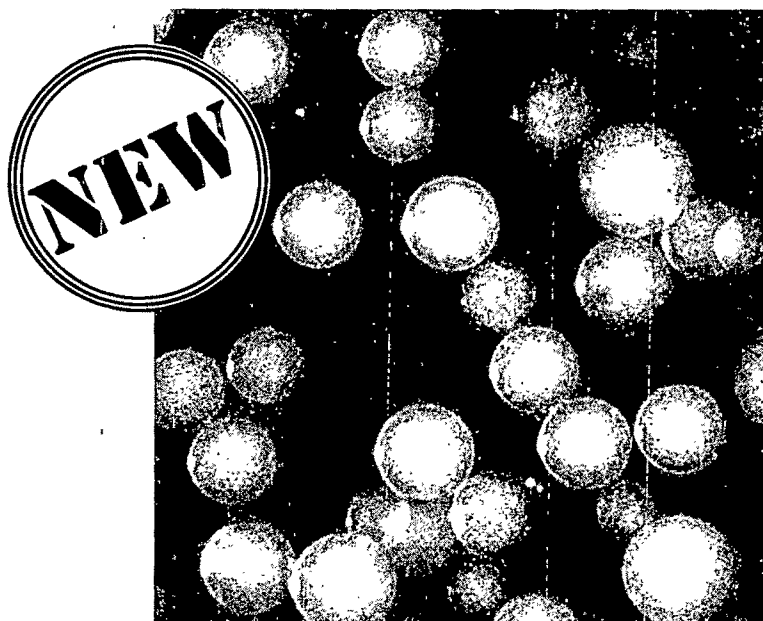


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Greek science today

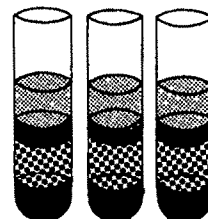
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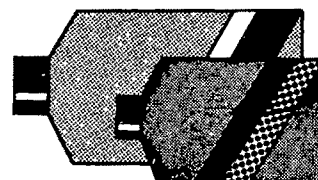
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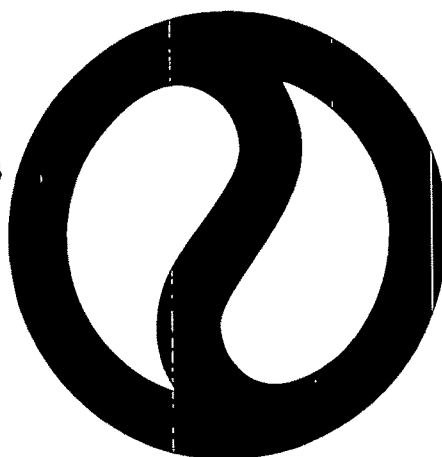


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Published weekly
ISSN 0028-0836

Registered as a newspaper at the
British Post Office

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Telephone: (01) 836 6633 Telex: 262024
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Pythagoras was one of the earliest of
the Greeks infatuated with truth for
its own sake. But what future for
truth? (page 391)

And what future for Greek science?
(page 394).

[Mary Evans Picture Library]

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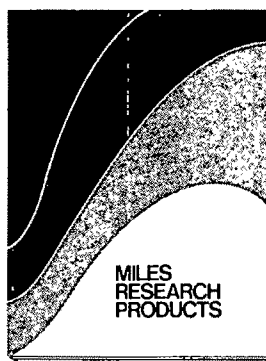
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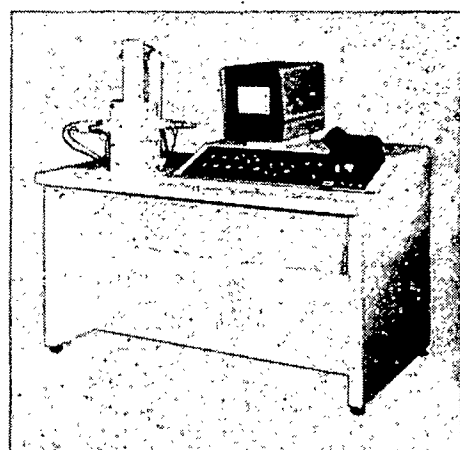
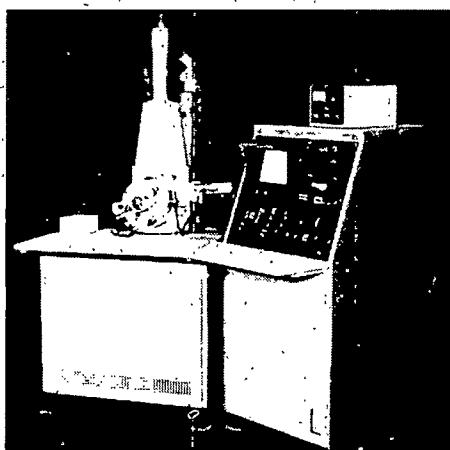
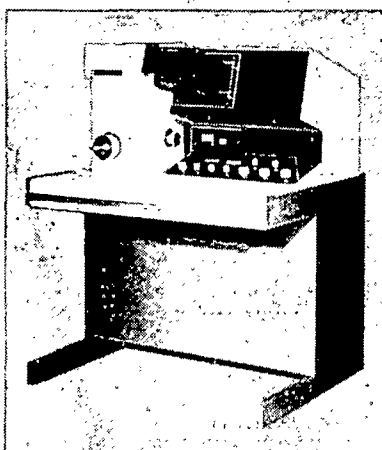
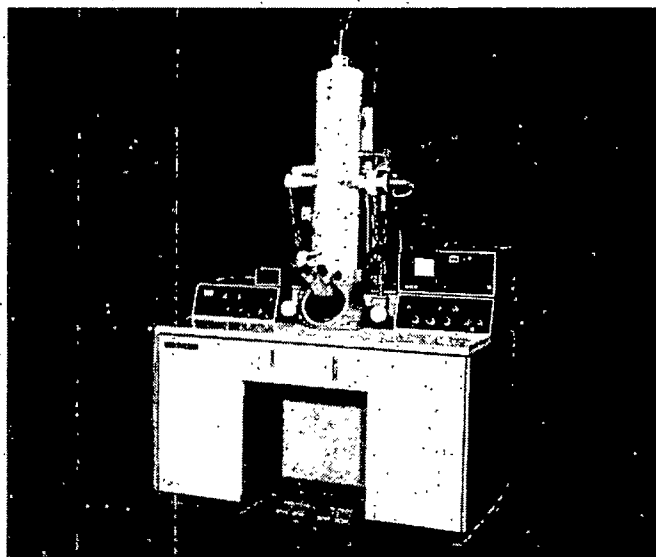
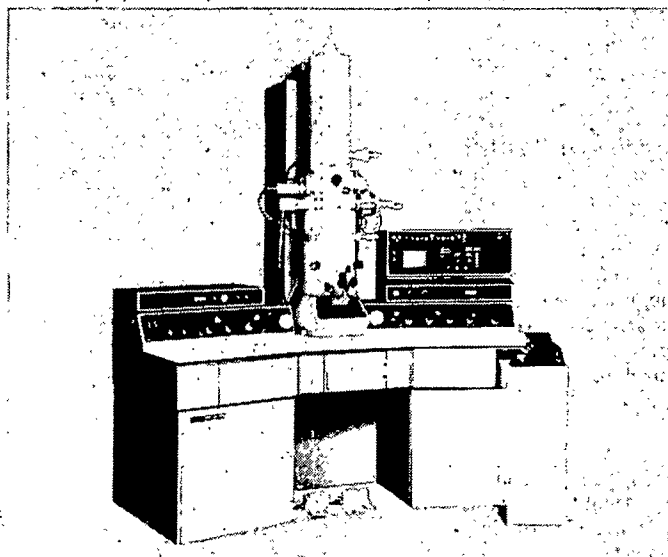
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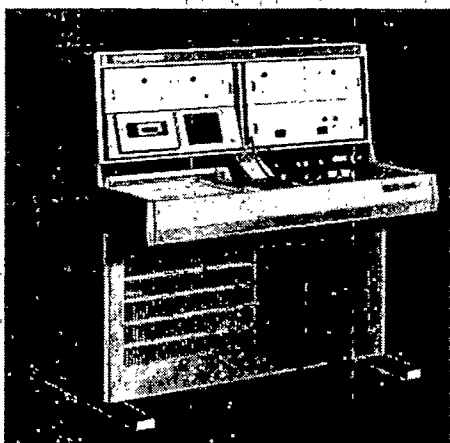
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Truth at any price

GEORGE STEINER, in his recent televised Bronowski Memorial Lecture, asked "Has truth a future?" For he fears that there may be a fundamental incompatibility between man's hopes of justice and decency and certain categories of truth.

Steiner, whose lecture is published by the BBC at £1, remarks that an infatuation with truth-for-its-own-sake emerged in Greece and Greek colonies between the seventh and fifth centuries BC, and must be clearly contrasted with the pursuit of useful knowledge—an occupation of many times and many places. "The man prepared to risk destitution or ridicule in order to solve a quadratic equation or a paradox in logic is an Ionian or south Italian Greek in the age of Pythagoras and Anaximander, of Heraclitus and Anaxagoras. Those who today pursue abstract truths in the sciences, in mathematics, in philosophy, are heirs to his hunger."

But, continues Steiner, abstract thought and the pursuit of truth have had their detractors, even enemies. Mystics and irrationalists, dogmatists and supernatural revelationists all in their different ways see mathematics, science and other scholarly pursuits as of a lower order than knowledge revealed through ecstasy or a divine statement. Then there are the romantic existentialists, Blake, Wordsworth, Kierkegaard, Tolstoy and so on, for whom 'the values of ecstatic self-realisation, of moral goodness, of simplicity, even of absurdity are set high above those of mathematical-scientific truth'. Finally, there are the truth-is-not-neutral critics who assert that truth and logic is used as a class weapon of oppression. There can be found mathematicians and scientists, Steiner might have added, holding with unmistakable sincerity all of the above views.

Now, however, we are confronting a new challenge of a rather different kind, he claims. Some of the truths that we turn up or might be capable of turning up could be in conflict with ideals of social justice or even survival. We know, as an extreme example, that sometime in the remote future the universe will run down in some way or other, and that will be the end of us. This piece of information is tolerable to most people and does not lead to any great move to restrict cosmologists' freedom of action. But some things are much more immediate. Predetermination of the sex of the foetus, cloning, the contributions of heredity and environment—such matters are the stuff of present-day research and the results of this research could have immediate implications for the social system we know. Many would argue that we are within sight of revealing verities of a kind that society cannot handle, and that now is the time for a socially responsive science, scaled down to human needs. "Not the costly elitist chase after the esoteric and possibly destructive fact, but the arcadian pilgrimage towards self and community."

But it won't work, claims Steiner; partly because nation-states have now come to believe that future grandeur and security might depend on unrestricted investigation, but mainly because "the obsession with objective and abstract truth is imprinted on the western mind", and even if truth lies in wait for us with hideous snares, there will always be those who will press on. Bronowski, humane and learned

man, might have said that "if man's endurance as a more or less decent creature depends on leaving certain doors closed, then so be it", thinks Steiner. But the other point of view, that truth matters more than man, is "also possible".

This is not unfamiliar territory; for instance last August Sir Andrew Huxley was asking similar if more specific questions about research into the heritability of IQ, and nuclear physicists have agonised over morality and research for more than thirty years. But it can rarely have been raised in a more penetrating and cross-cultural way. Yet for all that, it is possible that however carefully balanced and scrupulously worded Steiner's argument is and however agonisingly he has had to choose to come down on the side of truth, even against man, the picture that he has painted of the emotive concept 'truth' is altogether too stark, and the choice between man and truth made much too clear-cut. If one were to ask most scientists how they view their profession, they might well say something about hoping to add to knowledge, or conceivably might talk about making falsifiable hypotheses or learning by trial and error. But the word 'truth' might never crop up. Partly this would be because of the debasement of it by the propaganda and publicity world. But more likely it would be because the spirit of scientific enquiry tends not to reveal truths so much as to answer some questions at the expense of raising others of a more fundamental character. For sure a question such as 'is it true that the sex of a child can be pre-determined?' may well be answerable in the affirmative in the foreseeable future, but such a question is more one of technique not an eternal verity. Steiner persistently conjoins mathematics with science in his lecture, indeed uses mathematics for the examples of the elucidation of the earliest truths, and this may well be a mistake. The rigorous logical consequences of mathematical enquiry have very little in common structurally with the results of biological enquiry.

Nor is this the only ambiguity that comes from too stark a portrayal of truth. Those scientist who think seriously about marking certain doors 'too dangerous to open' are by no means all committed to "putting the questing, autistic brain to pasture, while instinct plays" or even to an "alternative style of cerebration" as Steiner would have it. They still do recognisable research, sometimes highly esoteric; they still submit papers to journals, live with peer review and hope to outpace their rivals—in short they behave like other scientists, and if truth were the right word for it, they would equally be reckoned to be pursuing truth, but not necessarily truth at any price.

Many years ago scientists recognised some sorts of experiments with humans and animals as ethically unacceptable. The doing of such experiments might yield all sorts of valuable knowledge but at an uncertain expense of suffering or degradation. So 'truth' was retarded, but biomedical researchers are not the less dedicated to the pursuit of knowledge for almost universally honouring this ethical code. We may at present be groping towards a similar code for the protection of society, and just possibly in a hundred years time our successors may deem the world of learning to have been little hurt by it. □

Saudi Arabia and the travelling scientist

ONLY in the past week has the outside world come to hear of the particularly savage public executions in Saudi Arabia last November of a princess and her husband for, apparently, contracting a marriage contrary to royal decree. At least the couple were spared being stoned to death—a fate which befell three men in Saudi Arabia last year after they had been found guilty of sexual offences. The Islamic code of justice has harsh things in store for sexual transgressors, and Saudi Arabia is not, of course, the only country that operates such a code.

But why is this any special concern of the readers of *Nature*? Certainly we should be exercised when states interfere with the elementary human rights of scientists or when they tamper with the means of scholarly communication. But is this recent matter, however barbarous and seemingly unjust by our different moral and legal standards, any cause for scientists to take special interest in?

The answer is yes. Saudi Arabia is in the throes of a mighty transition, often described as a leap from the Middle Ages to the atomic era. Science, technology and medicine are playing an enormous role in effecting this change, and higher education—non-existent before 1947—is now open to several thousand students each year. What is more, Saudi Arabian interests are not exclusively in applied science;

there has been talk of a particle accelerator and rather more than talk about a major telescope. All of this has meant an increasing flow of Western scientists into the country to teach, collaborate and advise.

Such scientists have an easy and relaxed access to the Saudi intellectual community—easier, probably, than that of any other group, as they are not there specifically to sell equipment, buy oil or represent the delicately balanced interests of their home country. They thus have an excellent opportunity for quiet and honest conversations with hosts about the way that Saudi Arabia looks from the outside world and how decades of impressive achievements can count for little if there are not comparable developments towards at least the elements of a society that is humane and just.

Much informed opinion in Saudi Arabia would probably already concur with all this, but its hand would be immeasurably strengthened in pressing for change were the message to be repeated from the outside as often as possible. And there are several other countries where, equally, the travelling scientist can be a power for good in this way. Maybe we underestimate the influence that the global scientific community could have with its liberal attitudes and ease of access to people of influence. □

Medical education and biomedical research: uneasy equilibrium

George A. Silver, Professor of Public Medicine in the School of Medicine, Yale University, argues that medical education should be separated from research if the best doctors and research workers are to be produced.

AMONG the casualties of the upheavals of the 1960s, one of the most far-reaching has been the dissipation of unlimited faith in the benevolence of science and the efficacy of progress along with a loss of confidence in social institutions generally. Biomedical research and medical education have both suffered the consequences, although in different ways and to a different extent. There is dissatisfaction with the medical care system, a dawning recognition that not all diseases may be curable and scepticism of the motives of both medical educators and researchers.

A medical school dean writes (Sherman M. Mellinkoff *Johns Hopkins Med. Jour.* 141, 167; 1977): "Triumphs of the biomedical sciences have burgeoned exponentially. Nevertheless there sometimes seems to be a miasma of something between apprehension and gloom over the American medical schools of this decade. Seldom has medical education been subjected to so much criticism, ranging from valid to vicious, from thoughtless to mindless. It is as though success had earned no laurels but a handful of nettles." And he attaches a litany of the public and popular complaints, among which the outstanding are "... that medical students are deliberately selected or molded to be overly scientific and lacking in altruism" and that the schools "... pay too much attention to abstruse problems and neglect common ailments ..." both of which he considers false.

In searching for the culprit, if there is any, the questioning seems to centre on this intertwined relationship, the apparently indissoluble bond, between biomedical research and medical education. A measure of obloquy has in recent years fallen on Abraham Flexner, the educator whose report on medical education in the United States (*Medical Education in the United States and Canada*, Carnegie Foundation for the Advancement of Teaching, NY (1910)) is considered the platform from which modern medical education was launched. Although Flexner epitomised a tide then running and so gave his name to the course of events already in train, he should only bear responsibility for recognising the trend and helping establish a rational base for medical practice. He did propose the university as the locus for medical education and medical research as an important associated activity.

But 'research' in the early years of this century was not the research we know today. One must visualise Sir William Osler, observing patiently and critically, recording the natural history, symptomatology and autopsy evidence of disease as the prototype of the investigator of that day. There was only modest and inexpensive equipment in the laboratory for examination and study. In short, 'research' was essentially clinical study in a scientific style.

Now that we have a multimillion-multibillion dollar research industry fastened upon the medical education process throughout the academic world, we begin to have second thoughts. Examination of the medical research and medical education budgets for 1975, shows that while \$800 million went specifically for research, only \$368 million went into teaching and training in the academic institutions. Total budgets for the medical schools were twice this amount, including many other kinds of payments, but

research costs are heavy elements.

There has never been any secret about the fact that the goals of the research community have been to use the medical educational establishment to further its goals. "The simply stated goal of the NIH has always been to develop a science base for the formulation and solution of medical problems" (James A. Shannon *Jour. Med. Educ.* 51; 1976).

For the proponents of the doctrine of the union of research and medical education, the course is clear. "If the definition of the goal of medicine as the saving of life, the relief of pain and the prevention of disability is accepted, it then follows that the tight coupling of biomedical science and clinical medicine is the critical feature of the medical school" (Donald W. Seldin *Clin. Res.* 23, 280; 1975). And dilution of this bond is reprehensible: "[the introduction of community services] has had the effect of changing medical schools more and more into second rate public utilities . . . compromised as institutions for the education of students in the biomedical sciences." If biomedical research distorts medical education and medical education is a constraint on biomedical research, should the two be bound together? The international implications are far-reaching, in view of the world-wide imitation of the American design.

There are other voices. In a recent conference, some influential medical educational leaders expressed concern: "There are many signs that society is finding the medical profession too insensitive to health care", stated one medical school dean. Students seem to be selected, educated, engineered toward goals of scientific excellence that are only remotely related to the everyday needs of citizens. The participants were told that only 35% of faculty time was spent in basic instructional activities and they wondered if more time couldn't be devoted to teaching in order to reduce the phenomenal faculty to student ratio in this country. Ronald Christie, a dean, professor and clinical practitioner on three continents in a review of medical education world wide (*Medical Education and the State* DHEW publ. no. (NIH) 76-943 (1976)) expressed some doubts about the almost one-to-one faculty student ratio in the US, also pointing out that in Canada the ratio is one-to-three; in Australia one-to-ten and in the UK one-to-sixteen. Since these countries seem to graduate excellent doctors, on a par with the US product, the discrepancy—and the expense—is puzzling. The cost of medical education, per student per year averaging \$12,650 in the US when studied by the Institute of Medicine of the National Academy of Sciences, and ranging quite widely, from \$6,900 to \$18,650 (*Cost of Education in the Health Professions* IOM (NAS) DHEW publ. no. (HRA) 74-32 (1974)) seems to be twice as high as Canada's per student cost; three times Australia's and five times that of the UK.

If cost alone were the factor, one might hardly quarrel with the result, since the US is notoriously lavish in its expenditures. But the Christie note on the value of research as a concomitant of medical education, with which he heartily concurs, also queries the calibre of the research that is too often done to satisfy the requisite standard without providing the product required. He calls the mandarins to order in his comment that "Few of those involved in research in academic departments will admit even to themselves that their research is pedestrian or unprofitable . . ."

And a university president states (David S. Saxon *Jour. Med. Educ.* 51, 991; 1976) in connection with the shibboleth of academic tie as the *sine qua non* for medical education: ". . . these opportunities (for mutual exchange of ideas and research advances between the medical and general campuses) are rarely exploited."

A science administrator points out (William D. Carey *Science* 197, 825; 1977) how the tight bond between the academic, medical, educational and research areas may be

damaging to research activity: ". . . built-in inefficiencies and distractions are sapping the vitality of the research process . . . the dollars allocated to basic science no longer tell us much about the true levels of the research effort."

More grave a charge is levelled by a social scientist studying medical ethics in the practice of biomedical research (Bernard Barber *et al. Research on Human Subjects*. N. Y. Russell Sage; 1973). He quotes Merton, "The culture of science is, in this measure, pathogenic. It can lead scientists to develop extreme concern with recognition . . ." And he himself adds, ". . . we have presented evidence that the pressures of having to establish oneself in a competitive scientific community seem to have the effect of making these researchers engaged . . . less sensitive . . ." And again, ". . . our findings make it clear that it has some negative consequences for other important human values . . ."

If this is the attitude encouraged among researchers, should it prevail among students of medicine? Today's medical education makes the doctors much more dependent upon the technology of medicine, not only serving to reduce contact with and interest in the patient as a person, but also generating enormous expense in medical practice. Reinhardt ('Health Manpower Policy in the United States'. Paper presented at the University of Pennsylvania Bicentennial Conference on Health Policy, 11-12 November, 1976. Quoted in Somers and Somers, 'A Proposed Framework for Health Care Policies'. *Inquiry* 14, 115-170, June 1977) estimates that each physician in the US is the source of \$260,000 of medical expenditure each year.

Few rational people in this century would want to dispense with important, tested and proven advances in medical science and practice; nor would they propose discontinuing the admirable research efforts of this and other countries. The question at issue is, need biomedical research be so intimately, inextricably bound to medical education? It is not irrational to ask whether the medical educational process could not be carried on separate from, though with useful liaison to, the biomedical research efforts. The changed emphasis would allow a different selection, admission and process, less emphasis on technology and more emphasis on the patient as a person. It might help to redirect physicians in their practice, reduce the volume and cost of medical care, without increasing the mortality or disability rates among Americans. To be sure, some are already questioning the efficacy of medical care in reducing morbidity and mortality in any case (John B. McKinlay and Sonja M. McKinlay *MMFQ/Health and Society*; 1977).

On the research side, the separation might be beneficial as well. Investigators, choosing a career in which they could advance without competing for academic title, would not be split between the burdens of teaching, for which they never have enough time, and the necessities of the research activity, for which more constant and undivided attention is also demanded. Less money would be wasted in 'routine' research whose sole purpose is justifying the medical educational/research mix. As the students' role-models change, medical-practice careers will change. As Barber says (*NEJM* 295, 939; 1976), "Medical students are bright and ambitious; they have no difficulty ascertaining which subjects are taken seriously by the prestigious senior members of the faculty".

Biomedical research and medical education need to be separated to save them both from becoming isolated from the realities of the social setting, condemned to declining support in a climate of hostility in what should be their natural constituency. There is need for special attention to both. A medical education that glorifies research while ostensibly preparing students for practice will provide society with neither the best doctors nor the best investigators. □

Greek orthodoxy

The first of two articles commenting on the research policy and universities of Greece by E M Pantelouris describes the patriarchal and bureaucratic structure of Greece's higher education system.

WHY, one may ask, do 30,000 Greek students, representing nearly 30% of the student community of Greece, study abroad? Is this a vote against Greek higher education? Or are there just too few places for too many students? To describe the system is an answer in itself, and I shall attempt to do that here.

The aspiring Greek student cannot rely on the education provided by his or her six years of secondary schooling to secure a university place. Entrance is by written examination, conducted uniformly throughout the country, and to prepare effectively for these examinations, secondary school pupils commonly attend classes run by private cramming schools, which are a growth industry in Greece. Quite often, a young man or girl will spend a whole year after coming out of secondary school attending these 'frontisteria' before attempting, or re-attempting, the university entrance examination. Pressure is high, as the 1977 figures illustrate: slightly over 13,000 places were available for some 78,000 applicants (see Table 1).

This first hurdle over, the successful candidate embarks on a degree course that lasts as a rule four years (more for medicine and for some branches of engineering). Degrees are, rather general, with six or more subjects continued to the fourth year. Lectures are formal and *ex-cathedra*, a format aggravated by the relative absence of a large body of published books in science subjects, and the extremely poor library facilities. Textbooks prepared by the professor in charge of each course are produced and distributed free by the government. This is very helpful, as few textbooks can be published economically in Greek, but might fossilise lecture courses.

There is nothing comparable to the British 10:1 student-staff ratio. No average figure can be given, but however calculated, the ratio would be several times higher. The professor of a 'chair' is the only one officially entitled to lecture to students, except for one or two 'authorised' sub-professors (*ifigitis*). The rest of the staff are kept incommunicado, except for the laboratory sessions. It has been put to me that they are selected as demonstrators rather than lecturers: most of them still have no higher qualification than a primary degree. Teaching is thus concentrated in the hands of the professor.

Dr E. M. Pantelouris visited Greece as a Nature travelling fellow.

Table 1 Entrants by examination to university level institutions†, 1977-78

Law*	2,896] total 6,036
Economics*	3,140	
Literature, philosophy	1,145] total 2,030
Theology	100	
Modern Languages	785] total 4,329
Scientific and agricultural		
Medicine, pharmacy, veterinary science	1,017] total 13,412

*About a third of law and economics students are gainfully employed and hence in effect part-timers. For comparison, in the UK about 30% (212,000 including 49,000 of the Open University) of students—including postgraduates—are part-time
†Excluding physical education, teacher training and military schools

Table 2 An estimate of the number of Greek students abroad (before 1976)

Student population in Greece at university level	80,000
Total of Greek students abroad*	30,000
Annual student entry at universities abroad	6,000
Distribution of Greek students abroad:	
Italy	55%
UK	14%
France	8%
Germany	6%
USA	6%
Others	11%

*Based on applications in 1975 for foreign exchange for the purpose of financing full-time studies abroad

Academics and bureaucracy

Academic staff are also utterly dependent on the professor for tenure. Appointment to an assistantship lapses after nine years, unless a doctorate is obtained—an invitation to professors to grant internal doctorates to some to save them from redundancy. After a further six years, it becomes necessary to submit another thesis for the title of *hyphigitis* (or 'dozent'), some of whom may eventually be entrusted with a course or part of a course. Laboratory facilities, and acceptance of theses for such in-service internal degrees, depend in practice on the discretion of the professor, and reflect more his decisions about continuation of employment rather than any independent assessment of research ability.

Unavoidably this archaic system lowers the level of undergraduate teaching and prevents intellectual communication between staff and student. The students' response is disenchantment with the system, and the short term attitude that one is there just to get a degree of sorts, as nothing else

can be got—unless it is the intensive politicisation reflected in the slogans that completely deface the walls of university buildings.

Of course, students and staff are aware of the shortcomings of the system, care about it, and agitate. And it must be stressed that this system is breached by commonsense in many departments, where younger professors begin to bring to bear the approach under which they themselves trained abroad in the Anglo-Saxon world or elsewhere. But there remains in all cases the stranglehold of the greatest scourge of Greece: bureaucracy. If a professor wishes to authorise his second-in-command to give a course of lectures, the move has to be approved by somebody 'at the ministry', who will first calculate whether the lecturing load of the professor entitles him to such help—because an honorarium will be paid. Similarly, after an annual grant has been allocated, the department still has to submit for approval a list of purchases before they can be effected; so that work can be delayed for months because a chemical cannot be purchased outright. I have come across cases where the professor pays from his pocket to save the situation.

Here we come to the real key obstacle to reform: the tradition by which university regulations are nearly uniform for all institutions, and acquire their force through the ministry and publication in the government *Gazette*; that is through a centralised extra-university and political organ. University independence is restricted; and in abnormal periods—of which fortunately the present is not one—professors are dismissed or appointed by the will of the minister.

Yet paradoxically, the key to improvement lies with the government. If matters were left to the ability of universities to renew themselves, the impression is that the older Athens institutions would remain static, although the outcome could be different at the newer University of Thessaloniki. In one institution with some 25 professors I was told that only a handful favour reforms. To understand these attitudes it has to be taken into account that the most influential groups of professors are those in fields such as medicine and engineering, where titles of professor, *hyphigitis*, and so on are springboards for lucrative private practices, consultancies, and membership of innumerable committees.

A law is promised to replace the 'chair' system with a so-called sector system. Students and staff understand this to mean independence of university teachers in matters of lecturing and marking in their speciality, choosing their own research and participating in the running of academic affairs. The long incubation of the law, however, is giving rise to suspicions that the sectors will emerge as nothing more than groupings of related chairs as before.

It is to be hoped that the government may in the end come down on the side of actual, not simply cosmetic, changes that might enable Greek universities to gradually become something beyond mass producers of graduates, and rise to be schools for researchers. This they are definitely not at present.

For example, facilities cannot be expanded speedily, under the current pressure for places, to achieve overall a better staff-student ratio and to make possible the general adoption of more useful working methods. It should however be feasible for science faculties to introduce research-oriented streams for their third and fourth year students. Entry into these streams would be by merit as judged by the student's performance in the first two years, and the numbers accepted could increase as more facilities become available. In this stream, subjects would be dealt with in depth, and tutorials, dissertations and research projects would replace set practicals and lectures.

Academic staff should be given a

greater role, not indiscriminately, but in recognition of higher qualifications obtained outside the employing department, that is, under present circumstances, in reputable centres abroad. Leave of absence for the purpose should be available.

Furthermore, non-professorial staff should be free, indeed encouraged, to seek, individually or in groups, research grants and thus to participate in government plans to stimulate problem-oriented research.

What has been said is sufficient to explain the annual migration of aspiring students. As undergraduates, they seek a place that was denied them at home. Naturally, most go where entry to a university is—or rather was—an uncomplicated and undemanding business. Thus more than half of them jump from the frying pan of the Greek into the fire of the Italian system. Even Romania has been recently invaded, and is now, like Italy, taking steps to reduce numbers (Table 2).

As far as postgraduate studies are concerned, it would be an excellent thing if Greeks went abroad so as to keep, on their return, national research within the mainstream of world science. In practice, the potential benefit to the country—and to themselves—is reduced by the haphazard acceptance of places anywhere, without due regard to the subject of the research degree, and the suitability of accepting institutions. However, despite its wasteful and unfair character, the process is creating a large pool of people trained in a variety of spheres. It is another matter whether Greece can

make rational use of them.

Reforms

Against this background, the determination of the former and the present governments of Mr K. Karamanlis to reform the educational system becomes better understood.

Compulsory school attendance is being extended from six to nine years, the first six in a primary school and the other three in a junior secondary. In this junior secondary, classics will be studied in modern Greek, thus allowing a shift of emphasis from grammar to content, and making an important saving of time for modern subjects.

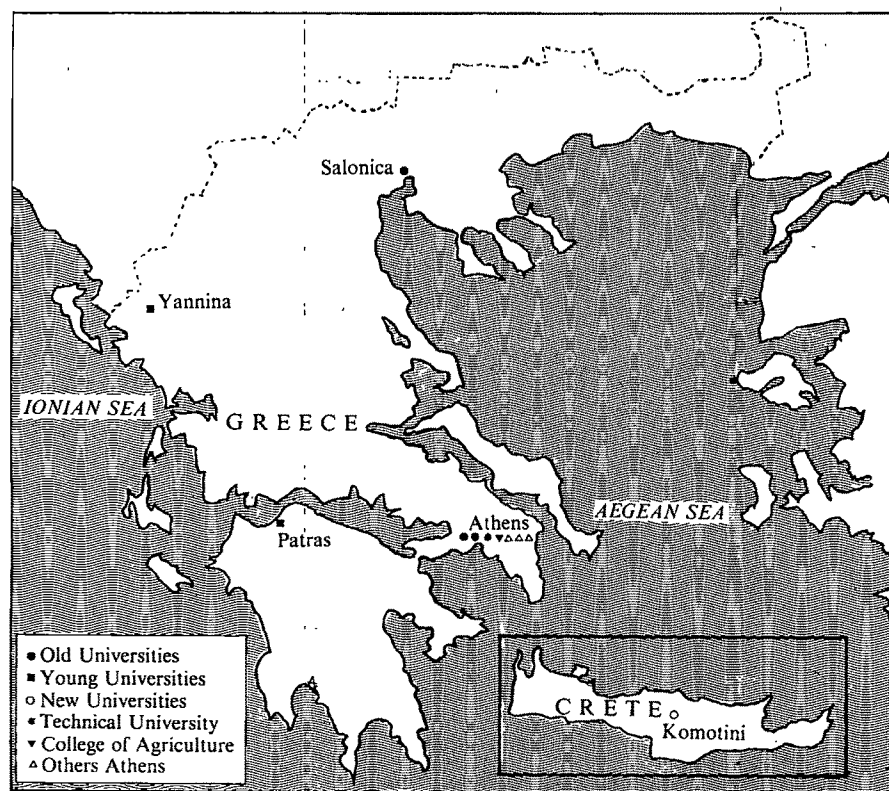
After completion of the junior secondary course, young people will sit examinations for entry to a senior secondary school of either an academic, or a technical-vocational stream. On emerging from the academic secondary, people will be entitled to a university place without entrance examinations (this will take effect as from 1980). Pupils who fail to gain entrance to either type of senior secondary will be enlisted for shorter vocational courses.

Putting such changes into speedy effect is no mean task. Change is proceeding apace, and is accompanied by the appointment of new teachers and the building of new schools at the rate of 1,500 classrooms per year. The demotic form of modern Greek has in the meantime been introduced throughout the educational system. New and better schoolbooks have been produced in record time. Investment of some \$250 million in technical education has just been announced by the government.

At the level of higher education, the young universities of Patras and Yannina are expanding steadily, and two new institutions are being established in Thrace and in Crete. There should, eventually, be sufficient places within the country for most qualified finalists of the senior secondary schools.

Some serious criticism can be made of the way in which the new universities are being set up. Under local pressure, the various faculties of the University of Crete will be scattered throughout the island, making impossible the creation of a coherent campus.

It is also unfortunate that planning of the new universities has been entrusted to detachments of professors from the old institutions. Although some of these people are very distinguished, the risk remains that the new establishments too will be made in the Bavarian style of the old ones. A unique opportunity of introducing creative diversification and innovation into the system will have been missed. Is it too late? □



Canada's nuclear visitor

"THERE are thousands of manmade objects in space. I suppose roughly half of them belong to the Russians and the other half to the Americans. . . . I know there are things up there. I know they are going to fall someday, but I don't want to hear about it until it looks as though they are going to fall in my country. When you hear that, call me again!"

Such was the breezy comment of Canadian Prime Minister Pierre Trudeau on the crash, or, to quote Montreal Radio, "unplanned re-entry" of Kosmos-954. This attitude seems typical of the understatement with which all parties concerned have treated the first cosmonuclear disaster, an attitude which *Pravda* described as "realistic" and "undoubtedly due to the climate of international detente".

Fortunately for the cheer-merchants, the errant satellite descended in an area where, according to Colonel Donald Davidson who led the search team "there is no specific indication" of people save "the odd trapper or trapper . . . a few solitary souls who normally work the area". The specific location of the debris "12.8 km northwest of the landing strip" about 300 km from Baker Lake and 1,500 km northwest of Edmonton brought home to the world at large that Canada had just abandoned the mile. Even the general vicinity of the impact was seized upon by the joke-makers ("Great Slave Lake, eh? Maybe it thought it was heading for the Gulag Archipelago!"). Even the fact that the satellite finally landed in an area with sufficient uranium deposits to give false readings on the search instruments was not without its special brand of irony.

But leaving aside both humour and grim speculation on the devastation which might have resulted had the

satellite fallen further south, a number of questions remain unanswered. Why did debris reach the ground at all, when, according to Tass, the nuclear power unit was designed to be fully destroyed and burnt on re-entry. And,



if the fail-safe construction of the craft was lacking in this respect, would such a burn-up have been, as claimed, without radiation hazard? How safe, indeed, is the debris? (The two members of the search team who found the impact crater have been removed to University Hospital, Edmonton, and the other members to the hospital in Yellowknife.)

More important, what is the procedure for notifying countries that a nuclear satellite may be about to fall? In spite of an opposition hammering, the Canadian Minister of Defence Danson and Secretary of State for External Affairs Jamieson seemed fairly content with the manner in which notification came largely through Washington. However, Canada

is a somewhat special case, being a participant in the US Space Detection and Tracking System via the optical facilities at Cold Lake, Alberta, and St Margarets, New Brunswick. (Canada is also associated with the USA in the NORAD early warning air defence system. Although this relates primarily to manned aircraft attack.) In return for this contribution to the tracking system, Canada automatically receives from the USA all relevant data on space objects. Hence the routing of the warning via Washington is at worst only a slight blow to Canada's *armour propre*. What, however, would have been the procedure if some other target country were involved? Would the message always go via "hotline" to Washington, or perhaps via the nearest "major" power? What then would be, for example, Dublin's reaction to such a warning being routed via London?

International agreements already provide for the search and disposal of crashed space-craft—the craft is to be returned to its owner, the target country reimbursed for damage and search costs. (Will the US participation in the Canadian search cause difficulties here?) What does seem to be lacking is a proper notification procedure, both of spacecraft in distress and spacecraft carrying potentially hazardous material. It would presumably not be impossible to extend the treaty banning nuclear weapons from orbit to nuclear fuel units, or failing an outright ban, to make all such "nuclear" launches notifiable to some central authority. Secretary of State Jamieson and Prime Minister Trudeau have already been discussing possible international talks on the problems arising from the aftermath of Kosmos-954. If these should be forthcoming and fruitful, one could well agree with *Pravda* that this unfortunate incident has, after all, turned out a triumph for detente.

Vera Rich

US agencies to study links between saccharin and cancer

SACCHARIN is to be investigated by the US Food and Drug Administration and the National Cancer Institute, who are to conduct an epidemiological study of 9,000 people in an attempt to determine whether there is a connection between saccharin and bladder cancer.

The dietary habits of 3,000 people diagnosed as having bladder cancer will be compared against those of a randomly-selected 6,000 controls. The study is expected to last 18 months, and to cost \$1.3 million.

Last November, Congress announced a moratorium on proposed legislation that would ban the use of saccharin

because of its suspected link with bladder cancer. Congress also agreed to allocate \$1 million for studies on the safety of saccharin, \$750,000 of which is for the epidemiological survey.

Congress's action followed the publication in October of a report on cancer testing technology and saccharin from its Office of Technology Assessment, which concluded that although there was evidence that saccharin is a potential cause of cancer in humans, there was no reliable quantitative estimates of the risk of saccharin to humans.

According to the OTA report, epidemiological studies of human experience carried out so far have not been sensitive enough to determine if saccharin is a carcinogen when injected. □

NSF encourages more university/industry collaboration

In a move carefully timed to coincide with the opening of Congressional hearings on its budget application for the year 1979, Dr Richard Atkinson, director of the US National Science Foundation, announced last week that the foundation plans a major expansion of its support for research projects carried out by joint university/industry groups.

No new funds are to be allocated in the NSF budget for such projects: they will be required to compete for support with other regular grants' applications.

Dr Atkinson said, however, that joint projects would be given more active encouragement than in the past, including a major publicity campaign aimed at both industry and universities. Industry was less willing than in the past to risk money on basic research and long-term goals, and was increasingly focusing on product improvement. "Given these trends, the nation is becoming more dependent than ever on universities and colleges to provide the basic research for new technologies and products," he said.

Last week's move follows past criticism from Congress that industry has not been able to compete for research funds on the same basis as university research scientists, a point heavily stressed, for example, by Senator Edward Kennedy during last year's authorisation hearings.

One outcome has been a recent decision by the National Science Board to amend its language covering applications from industry for such grants. Previously it had stated that these would only be considered under certain circumstances, namely if the research was of national importance, or if industry was able to provide unusual expertise or instrumental capability. The new wording is more positive, indicating merely the type of applications that will not—rather than will—be considered.

A particular point stressed by NSF officials is that the delay before pay-off from basic research is a deterrent to industry investing in this area, and that NSF funding might help redress the situation, for example, allowing those scientists who have joined industry because of a lack of jobs in more academic settings to carry out basic science projects.

This argument is not likely to carry much weight with industry, which has recently been telling the government that basic research is a federal responsibility, while development activities should be left to the private sector (a message reflected in the proposed shape of R & D funding within the President's 1979 budget request to Congress).

The sector which is likely to respond much more to the NSF's rallying cry are the small, knowledge-intensive, high-technology firms—such as those distributed around Boston's famous Route 128—many of whom have been experiencing financial difficulties in recent years.

Such firms, however, are rarely interested in basic research as such, even in the long-term. Their main concern—for which there is at present a lack of money—is finding ways of turning a good idea into a marketable product. And this is where, in practice, NSF support will be in greatest demand.

David Dickson

Argentina: US National Academy of Sciences to send delegation

THE Human Rights Committee of the US National Academy of Sciences is to send a small delegation to Argentina next month to investigate charges of repression against scientists.

The delegation, which will also visit Uruguay, will consist of Dr Christian Anfinsen of the National Institution of Arthritis, Metabolic and Digestive Diseases, Nobel Prize Winner in 1972 for work on the structure of proteins, Dr Robert Perry, of the Institute of Cancer Research in Philadelphia, and a member of the NAS staff.

During a ten-day visit to Buenos Aires and Montevideo, the delegation will talk with both scientists and government officials about scientific co-operation with the US, and about the situation of scientists in the two South American countries.

The NAS delegation's visit follows a recent trip to Argentina by Mr Emilio Daddario, president of the American Association for the Advancement of Science, and until recently director of the Office of Technology Assessment.

Mr Daddario said in Washington last week that the actions of the security forces in Argentina had, with a few exceptions, "made a shambles" of scientific freedom, and that reports reaching the West of repression against individual scientists were not exaggerated.

Referring to moves by western scientists to boycott the International Union Against Cancer congress, due to take place in Buenos Aires in October, Mr Daddario said that many of the scientists he had spoken to in Argentina felt it important to maintain contact with their colleagues in other countries, particularly since many of them lacked funds to travel to conferences and meetings abroad.

However he also said that government officials had asked him about the attitude of foreign scientists to the cancer congress, and that he had the impression that they were concerned to appear to maintain normal international relations with the scientific community.

Meanwhile moves are gaining momentum in the US to boycott the congress in protest at the treatment of scientists in Argentina since the army came to power in March 1976. Since that date, many scientists have been reported to have been arrested or abducted without formal charges.

Last month at a meeting of American Cancer Society Research professors in Phoenix, Arizona, all 39 present found that they were unanimous in their individual decisions not to attend the congress if it is held in Argentina as planned.

David Dickson

France falls out with Pakistan over reprocessing

PAKISTAN'S reaction to the latest move of the French government to modify the accord of 1976 on building a nuclear reprocessing plant, has been quite firm and unequivocal. In view of strong public statements there appears little chance that the Pakistani government will succumb to any retraction from the agreement, or soft-pedalling. A Foreign Office spokesman in Islamabad said that the government would not accept any change or modification in the agreement already reached.

The French want to substitute a co-processing plant for the planned plutonium reprocessing plant to guard against separation of plutonium that could be used in explosive devices. A co-processing plant can only reprocess the spent fuel once or twice for use in reactors and it does not produce plutonium. Pakistan's nuclear 'think-tank, however, has plans for using plutonium, particularly when it develops its fast breeder programme.

Mr Munir Ahmad Khan, the Chairman of PAEC (the Pakistan Atomic

Energy Commission), when asked for the rationale behind the timing of the setting up of the reprocessing plant, explained that PAEC wanted to synchronise the installation of the reprocessing plant with the commissioning of a few more reactors, but that for various reasons the reactor programme is running behind schedule. Mr Munir Ahmad explained, however, that the reprocessing technology is highly sophisticated and takes longer to set up than a reactor.

His team would like enough time to acquire the necessary experience in reprocessing technology for its optimum use. Hence, the hurry for the reprocessing plant.

At Chashma, the site of the proposed plant (plus a 600 MW reactor), the foundation concreting is in progress in anticipation of things ultimately working out as planned.

In his recent address to the Institute of International Affairs in Karachi, Mr Munir Ahmad explained the technical and economic reasons for building a

reprocessing plant in a country like Pakistan—which is planning on deriving almost half its energy supply from nuclear sources by the end of the century. Even the 2,000 MW from the big earth-filled Tarbela dam, he claimed, would be insignificant in filling the country's energy gap. And oil-fired power stations were becoming less viable, especially as Pakistan had so far struck almost no oil or good quality coal. So, the only option, according to Mr Munir Ahmad, would be the nuclear one.

His view is that in the overall nuclear fuel economy framework, reprocessing of used fuel plus plutonium is inescapable. Jacques Couture, sales manager of the reprocessing division of the French company, Compagnie Generale des Matières Nucleaires, has not missed his opportunity to blow the re-

processing trumpet. He has said that "with a typical light-water reactor which needs about 180 tonnes of natural uranium a year, the annual saving will be at least 40 tonnes, or even 70 tonnes if the plutonium recovered during reprocessing as well as the uranium is recycled." In fast breeder reactors, the fuel is some 20% plutonium oxide in uranium oxide, and the nuclear expert's view here is that a co-processing plant that cannot deliver plutonium is, therefore, not of much value, seen in that context.

Mr Munir Ahmad was vehement in his address on the topic of proliferation. He said that the safeguard imposed by IAEA had been fully incorporated in the agreement and that the French had imposed some additional safeguards. He saw no valid reason for the scepticism which had

been shown, and thought it might not be unfair to conclude that the motive for changing the agreement was to deny a developing country a useful, sophisticated technology of future.

Pakistan has sizable deposits of uranium and thorium-bearing minerals. Pakistani scientists are exploring the possibility of exploiting the indigenous sources of thorium in a thorium-uranium-233 cycle in fast breeder reactors, for which plutonium is essential.

The French have developed a proliferation-free uranium enrichment process—a chemical exchange method—but it is unlikely to be commercially viable for another decade or so. Likewise, the French concept of the co-processing plant has yet to be commercially accepted as it has not been tried in any other country.

Azim Kidwai

Europe's nuclear circus

PLENTY of shadow-boxing went on at last week's open discussions on nuclear energy organised by the European Commission, with no side quite hitting home but nevertheless a lot of energy expended. The proceedings were launched by news of an accident at one of Belgium's nuclear plants on 13 January. The accident was made public by the Environmental Protection Society who claimed that 80 people were contaminated by radium-131 and that the affair had been covered up by government authorities.

The news caused quite a scare in Belgium, but was ridiculed by speakers at the nuclear debate, doctors, and the director of the Tihange plant near Liège, where the leak occurred.

The so-called 'father of the H-bomb', Dr Edward Teller, a leading US nuclear physicist at the Hoover Institute for War, Revolution, and Peace, Stanford, California, told the nuclear hearings panel and an audience of several hundred that he was 'more alarmed' at the kidnapping of Baron Edouard Empain in Paris by terrorists, than by the Belgian leak. The Hungarian-born scientist claimed that he had been exposed to more radiation when he flew over Europe to be present at the hearings.

The state-run electricity company, responsible for the Tihange plant, put the figure of people involved at between 30 and 60. Director Robert Van den Damme said that no members of the public were involved during the accident which happened during an annual maintenance check. He said that workers ignored the leak until the job was done. Six people had

to be examined by doctors, who they said had been contaminated by 100 millirems of radium-131. They added that the maximum tolerated dose was 3,000 millirems for 13 weeks before the possibility of thyroid cancer occurred.

The Friends of the Earth argued that the principal issue was one of disclosure; that the authorities should have made the accident public and that their natural reaction had been to conceal it. But Mr Van den Damme denied that the affair had been hushed up. He said that the leak had not escaped from the plant and had therefore done no damage to the environment or the surrounding population. "It was not worthwhile to publish information about something that did not happen".



"If there is a leak, let's hope there are some terrorists in the area!"

The news did manage to give the Commission's nuclear hearings more relevance. The session ended after three days of heated debate between the supporters of nuclear energy and its critics. The highlight for many was described as a "confrontation" between two old debating partners, Robert Jungk, an Austrian futurologist opposed to the use of nuclear energy, and the controversial Dr Teller.

The theme of the session 'Economic growth and energy options: implications for safety, health and environmental protection', considered the problems of terrorist activities and the proliferation of nuclear weapons. Jungk argued that increased security measures to guard against the two problems could lead to centralisation and therefore police states. He said that this was a form of mental pollution that was not acceptable and advocated the immediate suspension of the nuclear energy programme.

Dr Teller hit back by describing what he termed as the worst possible pollution, that of poverty. He argued that the developed countries should devote their energy to developing nuclear power. He said that oil should be given to the third world to enable it to develop, whereas nuclear power was economically and structurally beyond its reach. And he put paid to terrorists by theatrically declaring that "the only way to get rid of terrorism is to get rid of terrorists".

The debate at least guaranteed a full house. EEC energy commissioner Guido Brunner made no final assessment of the findings of the hearings. Critics claim that there was never an intention of altering the Commission's approach to energy policy as a result of the debate.

Patricia Kelly

Polish censorship

COPIES of the Polish censorship regulations and instructions for the period February 1974–February 1977 have recently become available, via the unofficial "Social Self-Defence Committee" (formerly the "Workers' Defence Committee").

The subjects of banned articles during this period included: reports on industrial pollution of the environment, criticism of the bill on psychiatric medicine, information on the carcinogenic properties of PVC, discussion of the backwardness of scientific education in comparison with the West, errors in the selection of candidates for higher education, and data on the type of parasites afflicting Polish cattle.

The standing "Notes and Recommendations of the Central Office of Control of the Press, Publications and Entertainment" specifically prohibited publication of information on chemical hazards in industry and agriculture, work safety and hygiene, alcoholism, the Katowice mine disaster, and the purchase by Poland of production licences from capitalist countries.

Vera Rich

US soldiers in cancer study

THE US Department of Defense has promised to undertake a "crash programme" to examine the records of military personnel exposed to nuclear weapons tests in the 1950s to see if they provide evidence of any abnormal incidence of cancer. The promise was made by the deputy director of the Defense Nuclear Agency, to a House sub-committee on health and the environment during the course of public hearings last week on the dangers of low level radiation.

The sub-committee has been particularly concerned at evidence suggesting an abnormally high incidence of leukaemia among the 2,235 soldiers who took part in manoeuvres after a 45-kiloton test shot, known as Smokey, which was detonated in the Nevada desert in 1957.

A study of the possible carcinogenic effects of radiation on those involved is being carried out by the Center for Disease Control, whose director, Dr William H. Foege, told the sub-committee that the eight reported cases of leukaemia among the 2,235 was "out of the normal range".

Earlier Dr Karl Z. Morgan, who at the time of the test shot was director of health physics at the Atomic Energy Commission's Oak Ridge Laboratory, told the committee that his staff had been unable to retrieve scientific measuring equipment after

the shot because of "residual contamination". He had "no doubt whatever" that this radiation had caused the leukaemia now found in those who had taken part in the manoeuvres.

Dr Morgan, who is now a professor at the school of nuclear engineering of the Georgia Institute of Technology, criticised scientists who refused to accept evidence that there is no safe level of radiation, and that any dose, however small, can cause leukaemia and other forms of cancer.

"One of the many problems we face today is that many scientists have accepted the threshold hypothesis as a cardinal law and have lived with this hypothesis so long that they have become staid or petrified in their thinking. Now they cannot believe or accept the fact that the threshold hypothesis is wrong", Dr Morgan said.

David Dickson

Brothers in human rights test case

THE cyberneticist Gol'dshtein brothers—Grigorii and Isai—of Tbilisi, first hit the "human rights" headlines in 1975, when their exclusion from the Tbilisi Conference on Artificial Intelligence led to a confrontation between the international conference committee, who urged their admission, and the Georgian hosts. The brothers are now, once again, involved in what may prove to be a test case—Grigorii has been arrested, and Isai has been threatened with arrest on the charge of parasitism and "malicious evasion of socially useful work".

The charge of "parasitism" (being without visible means of support) was brought last year against refusenik Iosif Begun, who was sentenced to two years exile in Siberia. The authorities refused to acknowledge that he had

any means of livelihood, since his current occupation—teaching Hebrew—was not a recognised profession. The Gol'dshtein brothers, however, until their dismissal from the Mendeleev Institute of Metrology in 1971, had been earning 600 roubles (£300) per month each. Since the minimum wage in the Soviet Union is 60 roubles per month, the Gol'dshteins have managed to convince the authorities that they have been living on savings lawfully laid by from their earnings. Nevertheless, it is claimed, they are evading their responsibilities by failing to undertake socially useful work.

Both brothers were dismissed from their Institute in the routine manner, following their application for an emigration visa—an application which was refused on the familiar grounds of access to secret information. Unlike most refuseniks, they have now been "offered" jobs commensurate with their academic status, Grigorii in October 1977 and Isai in January 1978. These jobs, however, are at the "closed" Institute of Explosives Research in Tbilisi, where all work is potentially secret, and both in turn have refused to accept the offers.

On 17 January, Grigorii was arrested, and was released ten days later on a written undertaking not to leave Tbilisi. His trial is expected "perhaps in two to three weeks". On 19 January, Isai received an ultimatum from the police, that unless he finds a job within a month, he too will be arrested.

The Gol'dshtein brothers are so adamant in their desire to emigrate to Israel that in 1972 they unilaterally renounced their Soviet citizenship. Clearly, they are hardly the loyal comrades one would expect to find working in a secret institution. The jobs proposed for them are, they feel, yet another means of preventing their emigration.

Vera Rich

New threat to helium reserves

A demand that "all appropriate measures" be taken to conserve rapidly-diminishing supplies of helium for potential future use has been made in a report published last week by a committee of the US National Academy of Sciences. The committee, which was set up last autumn to provide the administration with independent advice on the politically-sensitive issue of helium conservation, suggests that there is "a strong case for building a substantial government-owned strategic reserve of helium". It also condemns the present venting of separated helium into the atmosphere by natural gas-producers.

Most scientists know helium merely as the second lightest element. Some have used its properties as a gas to fill meteorological balloons; others have more recently exploited its superconducting properties at temperatures close to absolute zero—where it remains in liquid form—for experiments ranging from nuclear fusion to the superconducting magnets that will be used in Fermilab's new energy saver/doubler.

But although current applications remain relatively limited, helium has become a major headache for the US government. Reserves of helium-rich natural gas, from which helium can

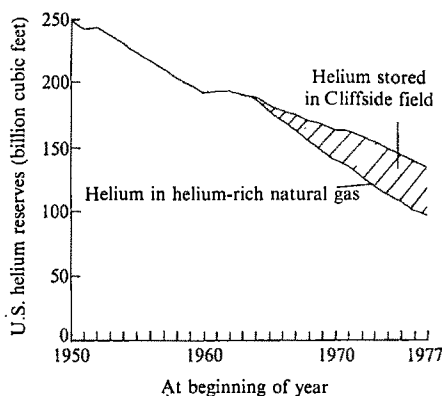
be extracted many times more cheaply than from air, are known to be very limited and, at current extraction rates, rapidly diminishing.

Awareness of a potential shortage of helium dates back to 1960 when the administration, with one eye on future federal needs for both defence and space research projects, passed a Helium Act. Under the terms of the act four companies were contracted to separate helium for storage from natural gas; the conserved helium was stored in the partially depleted Cliffside natural gas field near Amarillo in Texas, which at present contains about 39 billion cubic feet (bcf) of the gas.

But the act turned out to be an embarrassing and expensive mistake. With the run-down in both defence research and the space programme at the end of the 1960s, demand for helium dropped dramatically below expected levels—by 1971 the total market demand for helium was only half what had been predicted—with no indications that the situation would improve in the near future. The government's problems were compounded by the fact that private suppliers were able to offer helium at much lower prices than the administration.

The net result was that sales of helium by the Bureau of Mines in 1971 were lower by a factor of six than had been predicted. And the massive federal investment in conservation measures had resulted in an annual bill in interest charges alone of over \$20 million, with sales averaging only about \$1 million. One reaction was the immediate termination of the contracts with the four private suppliers—action which has resulted in lengthy litigation still making its way through the courts. Since then, natural gas producers have been venting helium either directly or indirectly into the atmosphere. And there have been growing demands from Congress that the whole issue of helium conservation should be looked at more closely, with an eye to new legislation replacing the 1960 act.

Last September the appropriation committees of both the Senate and the House ordered that a study of the present situation, with recommendations about possible new legislation, be carried out jointly by the Department of the Interior's Bureau of Mines and the Department of Energy (then the Energy Research and Development Administration). In their turn, these two agencies, which had also brought in the Department of Defense and the National Aeronautics and Space Administration (NASA) as two of the largest helium-users, decided that an assessment of the situation should be obtained from an independent non-



government organisation, namely the National Academy of Sciences.

Last week's report is the result of this study, carried out in only a few months by a specially constituted committee of the National Research Council (and taking into account the results of a public hearing on helium conservation measures held in Washington in November).

The committee's report is unequivocal and, at times, outspoken. "Obscured from public view by current dilemmas associated with energy supply and an unstable economy, public policy for the management of the nation's resources appears inadequate and inflexible" it says.

The committee points out that almost 96% of the world's resources of helium-rich natural gas is to be found in the US, and that with current rates of extraction, these are expected to run out within 20 or 30 years. Furthermore although current annual demand is only about 1 bcf, this figure is likely to be considerably higher by the middle of the next century, with potential applications in fields such as fusion and gas nuclear reactors, laser-based missile defence systems, and advanced energy conversion cycle.

"It is clear that even the maximum possible helium conservation programme will only buy time in which to adjust to the extraction of helium from air as the sole source" the committee says. It agrees that as a result of earlier conservation policies there is at present enough helium stored to meet federal requirements into the next century, but writes that "the case for helium conservation is long-term based on the interest of future generations . . ."

The report also points out that there is, at present, little market incentive for conservation of helium by private producers. It therefore suggests that the government contemplate taking "an aggressive role to resolve the legal and market uncertainties currently discouraging helium conservation."

Although the conclusions of the NAS report coincide broadly with the views of the government agencies

which commissioned it, they are likely to put a more subdued case in their own report to Congress, which is expected to be delivered within the next few weeks. Administration officials agree, for example, on the need to stimulate private industry's interest in helium conservation—but they feel that by concentrating on the the situation of supplies of helium-rich gas, insufficient attention has been given to the potential for extracting helium from ordinary natural gas.

Furthermore there is a feeling that the NAS report may have been too optimistic in its predictions of future demand for helium. For example, the report quotes a prediction by the Argonne National Laboratory that by the year 2050 the annual requirement for helium from fusion reactors and superconducting energy storage alone will be between 10.4 and 15.4 bcf. In contrast, information gathered from government agencies indicate that consumption in 2030 will probably be between 3 and 8 bcf.

The agencies, which have been asked by Congress for advice as to possible government action and future legislation, are therefore unlikely to recommend the renewed stockpiling of helium, at least not in the near future. Dr Ray Munnerlyn, who is responsible for helium policy at the Department of the Interior, said last week that in the present circumstances, he would personally be opposed to a recommendation that Congress should renew its helium purchases.

David Dickson

Erratum

We would like to apologise for the errors which slipped into the article 'Dioxin meeting recommends cancer study' on page 202 of 19 January issue. They resulted from dictation over the phone and subsequent editing and are amended as follows. The first sentence of the first paragraph should have begun: "Since the release of the tetrachlorinated dibenzo-p-dioxin (the isomer 2,3,7,8 tetrachlorodibenzo-p-dioxin) . . ." The company referred to as the Doll Chemical Company is in fact the Dow Chemical Company and Dr Rodolfo Saracci is at the Unit of Epidemiology and Biostatistics, not Immunology and Biostatistics as reported. The two groups from the University of Wisconsin and the Dow Chemical Company, which the article claimed reported animal studies to test the carcinogenicity of dioxin, were not in fact present at the meeting; their work was discussed in their absence.

correspondence

Catastrophe theory reply

SIR,—This is a reply to a number of recent letters (*Nature* 270, 381–384 and 658; 1977) attacking our article (Zahler & Sussmann *Nature* 269, 759; 1977) on applied catastrophe theory. Many of these cite applications in the physical sciences to rebut our criticism; but as we were careful to state repeatedly in our paper, our discussion applied only to models in the biological and social sciences. In a more specific complaint, the respected mathematician John Guckenheimer asserts that we ignored recent work of Kozak and Benham on denaturation (Benham & Kozak *J. Theor. Bio.* 63, 125; 1976), where the Maxwell convention, not the delay rule, is used. The effect of this change, however, is to remove the cusp, and thus the catastrophe theory, from the analysis. Dodson's letter gives another reason why catastrophe theory should not apply to denaturation.

A number of writers defend Zeeman's embryology paper (Zeeman *Lectures on Mathematics in the Life Sciences*, 7, 69; 1974). The meaninglessness of this paper's "first-order" quantitative results will be explained in Sussmann & Zahler *Synthese* (in the press). Guckenheimer complains that our discussion of evidence is misleading. We printed Zeeman's statement verbatim and then five facts which no one has disputed (with one minor exception; see below). Despite Guckenheimer's attempt somehow to separate confirmation of a model from confirmation of that model's predictions, we feel that most readers will agree that our assessment, and not Zeeman's, is supported by the facts.

Zeeman writes that our article ignores a rigorous version of his main theorem published elsewhere (Zeeman *Proc. Int. Cong. Math. Vancouver* 2, 533; 1974). We criticised the first proof for unjustifiably singling out the time-axis. The second proof adds an assumption, not mentioned in the paper addressed to biologists, which declares that the time-axis is special—postulating what cannot be proved. Ignoring all the other faults we found in his proof (which are not repaired by the new assumption), Zeeman concentrates his defences on one of our minor criticisms, which concerns the direction of curl of the isolated neural plate. In rebuttal we note that it is Zeeman, not ourselves, who is misquoting Crelin, who clearly states that the graft curls

laterally (Crelin *J. Exp. Zool.* 120, 547; 1952 and private communication); that the mesoderm is too thin to exert the forces Zeeman ascribes to it; and that 'neurulation', the subject of the section containing Zeeman's statement, includes Harrison stage 23, which Crelin studied.

Returning to Prof Guckenheimer's letter: he disagrees with our definition of the word 'catastrophe'; but the definition we use is that used by all the applied catastrophe theorists that Guckenheimer is defending. We agree with Guckenheimer's statement that the confusion of the intuitive notion of jump with the mathematical notion of jump discontinuity is common and useful. What we object to are attempts to switch from one to the other and back in mid-proof. Finally, Guckenheimer complains that our remark about the large number of unrefereed applied catastrophe theory papers is "snide". First of all, it is a fact. Consider, for example, the bibliography prepared by the strong catastrophe-theory supporter Lynn Steen in February, 1977 (Steen: *Catastrophe Theory: A Selected Bibliography* (mimeographed, 1977)). Approximately 70% of the papers classified there as applied catastrophe theory appear to be unrefereed; we think that this is an unusually high ratio for scientific papers. While no one would say that it is wrong to publish an unrefereed paper, we think it is quite proper to point out this phenomenon, because it indicates that catastrophe theory has grown in an atmosphere largely sheltered from outside criticism, and this partly accounts for its exuberant claims.

RAPHAEL S. ZAHLER

New Haven, Connecticut

In support of boycotts

SIR,—Richard Peto and Sir Richard Doll raise an important and difficult issue in their letter about boycotts of congresses to be held in countries controlled by repressive regimes (1 December, page 384). As one who lived and worked in South Africa until government action forced me to leave, I can say something about this topic from 'the inside', as it was a subject often discussed by those of us who wished to see change and about which conflicting views were held. I emerged from these discussions as a strong proponent of boycotts for the following

reasons (most of which form answers to the points raised by Peto and Doll):

- It is often argued that boycotts cause harm to the people one least wishes to harm—opponents of the regime, scientists who need "contact with the international community" or, in the case of economic boycotts, the mass of people of the country (for example, the blacks of South Africa). The fallacy in that argument is that most genuine opponents of repressive regimes will already have been dealt with by the government—imprisoned, silenced by banning, or even murdered. In most countries scientists can, and do keep contact with the rest of the world by travel and are not restricted unless they are actively engaged in politics or openly express opposition to the regime; if they do, they are likely to be dealt with as above. And, in my experience, a majority of politically-aware blacks in South Africa would have accepted the damage that might have resulted from economic sanctions as a necessary and effective means of putting pressure on the government.

- Visits to these countries by prominent people or holding of international congresses is often regarded as tacit acceptance of the regime by the rulers and sometimes exploited as evidence of its acceptability. To opponents of the regime such actions bring a sense of disillusionment and cynicism, and I recall the moral encouragement and gratification one felt whenever a prominent artist, scientist or sportsman announced publicly that he would not visit South Africa.

- Peto and Doll imply that scientists should be "apolitical" as this confers on them a special "useful image", presumably to be used for protests, such as that made by so many scientists against the imprisonment of Mikhail Shtern. The argument may be used with equal force by doctors, sportsmen, artists, businessmen or any other person pursuing any other occupation! No group is immune from the responsibility to take a political stance at times, whether this is expressed as a letter of protest or a boycott.

- Boycotts are often said to be ineffective and therefore not worth supporting. This is only true when they are not fully supported. The sporting isolation of South Africa has been most effective and has led to removal of many of the barriers imposed in that country against mixed-

race sporting activities.

● Would-be visitors to these countries often say they go to see and hear things for themselves. Indeed, I have often been asked to provide introductions to black opponents of South Africa's regime who could provide visitors with their side of the story. I reply that most genuine opponents have already been silenced one way or another and I would certainly not put at risk those who had escaped the net of repression by encouraging them to speak out to inquisitive strangers. I have known many doctors and scientists who have visited South Africa in the past ten years; most return to this country with new arguments and convictions, if not fully in favour of apartheid, at least condoning or "understanding" it, now that they've "seen the problem" for themselves (or been indoctrinated by their proselytising hosts?). Some come back encouraged by a few whispered sentiments of distaste for the regime, mistakenly to believe that there still exists in that country a substantial courageous body of doctors or scientists who oppose apartheid and are doing something actively about it. Not one of my colleagues has met a black person socially, and none has spoken out against the regime while he was there ("How could I say anything when they were my hosts?").

Clearly, one could carry such convictions to absurd lengths. Some degree of repression exists in most countries; minority groups often appear to be singled out and treated badly. Where one draws the line is a matter for individual decision. What I'm saying is simply that if one feels strongly enough about a situation in a particular country, there are good arguments in favour of staying away—and of making one's reasons known.

R. HOFFENBERG

The University of Birmingham, UK

Sauce béarnaise

SIR,—Perram, Nicolau and Perram recently described a method for reversing what they called coagulation of *sauce béarnaise* by addition of extra vinegar (*Nature* 270, 572–573; 1977). They stated that other authors recommend discontinuation of the 'experiment' of producing *sauce béarnaise* when coagulation occurs.

Amateur, non-mathematical cooks, such as myself, know that two kinds of deviation from homogeneity can occur during the preparation of *sauce béarnaise* and similar sauces such as *sauce hollandaise* (see Elizabeth David's book, *Summer Cooking*, first published by Penguin in 1955). The colloidal suspension can separate into its components, no doubt due to the forces

described by Perram *et al.* I have found, however, that sauce which has failed in this way can be made homogeneous quite simply by the addition of a few drops of warm water with steady stirring. This has the advantage of maintaining the delicate flavour balance, which would be disturbed by the unnecessary addition of extra vinegar. The second deviation from homogeneity occurs when the temperature of the sauce rises too high: the egg yolk becomes coagulated—as Elizabeth David notes—and the sauce becomes grainy in texture. This process cannot be reversed by the addition of acetic acid and it is to this event which culinary authors are referring when they recommend discontinuation of the 'experiment'.

ROY JOHNSON

Cambridge, UK

SIR,—We welcomed the long overdue treatise by Perram *et al.* on the stability of the colloidal suspension colloquially known as *sauce béarnaise*. However, as the authors claim primacy in reporting their successful attempt to resurrect this sauce by employing the theory of the stability of lyophobic colloids, we feel compelled to mention a previous notation in this field—Child, J., Bertholle, L. & Beck, S. *Mastering the Art of French Cooking* (Knopf, New York, 1966). The use of *lemon juice* here substitutes for acetic acid.

G. STEARNS

R. WEININGER

New Haven, Connecticut

SIR,—A professional biologist (and amateur cook) is amazed that Perram, Nicolau and Perram could by consideration of the theory of stability of lyophobic colloids deduce that lumpy *sauce béarnaise* could be made smooth by the addition of acetic acid and confirm it in practice. The biologist would not call the lumpiness coagulation, which to him is an irreversible reaction (Chambers Dictionary of Science and Technology) though the chemist may. The biologist would also control his experiment by addition of acetic acid solution without the acetic acid, i.e. water. This, reducing ionic strength, the practical cook has done with equal success for decades, though this 'know-how' is seldom described in manuals of gastronomy.

Furthermore, the first step in preparation of *sauce béarnaise* is a drastic 'reduction' by heat. Does not this remove most, if not all, of the original volatile acetic acid molecules before the addition of the fats? If so, the charge on the fat droplets must be provided not by acetic acid molecules, as postulated, but by less volatile acids

of the herbs and vinegar—impurities to the chemist but quintessential factors to the gourmet and variable damn-nuisances to the cook.

J. F. LOUTIT

MRC Radiobiology Unit,
Harwell, UK

SIR,—Perram *et al.* are to be congratulated on their attempt to define the underlying physico-chemical principles of the unstable system known as *sauce béarnaise*, and in particular on their published observation that addition of vinegar (acetic acid) will resurrect the sauce.

However, I must point out that my wife has adopted this practice for some years now on the very rare occasions when the sauce has coagulated. No doubt, other good cooks have done the same: cooks, however, unlike scientists, usually prefer to keep their knowhow secret rather than divulge it.

T. S. TWEEDIE

Chester, UK

Calculating the fine structure constant

SIR,—I would like to cite two references *Physics Today*, 24, 8, 17 (1971) and 24, 11, 9 (1971), in which reports have been published of attempts to calculate empirically the fine structure constant, whose experimental value in physics is 137.03602.

In these papers, the authors have attempted to calculate the value of the constant with pure members and π . The results are all very complex, and none are in exact agreement with the experiment.

I would like to propose a much simpler solution whose value is in exact agreement with the experiment.

$$137.0360157 = [137^2 + \pi^2]^{1/2}$$

Since this expression is very simple, it must be the true solution.

THOMAS J. BURGER

3M Company, Minnesota

Bright minds not wasted

SIR,—Your editorial (24 November, page 287) on the dearth of scientific appointments was immodest, probably unintentionally. "How do we get ourselves out of this bind, ensuring that bright minds are not wasted?", is the sentence that offends. I for one do not feel that my students will be necessarily accepting second-best if they do not become professional scientists.

The tenor of the article was antagonistic to the idea of the best minds going into other fields. Is it not this attitude which has made it necessary to force such a change for the good of the larger community?

S. D. DOVER

University of London, King's College,
UK

news and views

Chemical waves in *Drosophila*

from Jonathan Slack

OTHER sciences may progress by conjecture and refutation but this can hardly be said of contemporary developmental biology. Here progress occurs by the successive formulation of models whose quality is judged, at least in the first instance, by the amount of applause from the audience when they are first unveiled.

1978 has started well with a new model from S. Kauffman, R. Shymko and K. Trabert with what amounts to a general theory of development in the fruit fly *Drosophila melanogaster* (*Science* **199**, 259; 1978). The new model does nothing less than account for the relative positions of different parts of the organism and the assignment of the correct intrinsic characteristics of each part.

To understand what this means we must remember that most of the adult fly is formed during metamorphosis from groups of cells called imaginal disks. The rudiments of these disks are laid down in early embryonic development and during the life of the larva they grow but do not visibly differentiate. It has recently been shown that during their growth the disks become subdivided into compartments (Garcia-Bellido, Ripoll & Morata *Devl Biol.* **48**, 132; 1976). These compartments have the property that once the boundary has formed, the cells from the neighbouring compartments can never cross it, so each develops by further subdivision but not by recruitment of cells from outside. Certain homeotic mutations result in the partial or total conversion of one compartment within a disk into another. For example, the mutant *engrailed* converts the posterior compartment of each of the thoracic structures (wing, legs, haltere) into the anterior compartment. (Lawrence & Morata *Devl Biol.* **50**, 321; 1976). It is now widely believed that the compartment boundaries are the thresholds between the on and off states of the wild type alleles of what Garcia-Bellido calls selector genes; in which occur the homeotic mutations.

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Another property of the imaginal disks is that they can be cultured in the abdomens of adult flies for long periods of time and then forced to differentiate by implantation into a larva about to undergo metamorphosis. In general their state of determination (leg, wing, genital, and so on) is conserved during culture, but occasionally a so-called transdetermination occurs whereby a certain type of disk changes to another state of determination. Several of these changes are also mimicked *in vivo* by homeotic mutations.

Kauffman has previously published a most persuasive analysis of this and related data from which he concluded that the states of determination of the disks are given by a combinatorial 'epigenetic code' of 'on' and 'off' states of the selector genes (*Science* **181**, 310; 1973). A mutant selector gene will behave as though it were off in any combination and so may change several disks or compartments to different states of determination.

So, the development of the adult fly involves first the regionalisation of the embryo into a number of territories which will become the various disks and perhaps also the larval segments, and second a subdivision of each of the disks into its compartments. We wish to know how these subdivisions can be carried out to yield the correct combinations of selector gene activity in each compartment, and how the positions of the compartment boundaries, or thresholds of gene activity, are determined. The new model proposes an answer to these questions and it does so by bringing a new actor onto the stage to join the gradients and thresholds which usually play out the drama on their own.

Dissipative structures

This is the dissipative structure, the theory of which has been developed over the past 30 years by the able team of physical chemists in Brussels (see *Self Organisation in Non-equilibrium Systems*, Nicolis G. & Prigogine I., 1977). A spatial dissipative structure is a stable pattern of chemical concentrations distributed in space.

Such phenomena are called 'dissipative' because they can only arise in systems which are maintained far from thermodynamic equilibrium by a continuous flux of matter through the network of reactions.

This situation may seldom be encountered in the test tube but is of course the norm for biochemical reactions in the living cell. Dissipative structures are possible for coupled pairs of reactions which have particular relations of auto and cross catalysis, and although most of the work on their properties has been theoretical, several of the predicted dynamical features are realised in the Belousov-Zhabotinskii reaction (Winfree *Science* **175**, 634; 1972).

Drosophila model

As far as the *Drosophila* model is concerned the essential feature of this type of reaction network is that many dissipative structures may be possible for the same system. As a controlling parameter is varied the system will pass through a predictable sequence of spatial patterns. The conditions for the transitions can be established by the mathematical techniques of bifurcation theory which can indicate the critical parameter values at which the homogeneous state ceases to be stable to thermal noise, and which particular patterns present in the thermal noise will be selected for amplification.

Consider a straightforward example where the reactions are proceeding in a tube of length L . If L is small enough only the homogeneous state will be stable. Now imagine that the length is gradually increased. Above a critical value L_1 , the homogeneous state will cease to be stable and will be replaced by a monotonic gradient. At a greater length L_2 , the homogeneous state will be re-established. At L_3 , a second pattern mode will appear, which is a distribution of concentration of \wedge or \vee form. At L_4 , the second mode will revert to homogeneity. At L_5 , a third mode will arise, of the form \sim , and so on. In the general case there may not be a unique final outcome for a given length, but in the *Drosophila*

model there is a separate interval of length for each successive mode and the rather deep mathematical problem of predicting intermode transitions is avoided.

Suppose now that the reactions are proceeding not in a tube but in a growing line of cells. The cells contain switches which will turn on the selector genes when the concentration of the dissipative structure reagent exceeds a certain value. We have to assume that a different switch becomes responsive each time a new pattern mode is established. We further assume that the threshold values for all the switches are near the nodal values of the patterns, that is the concentrations which are the same as those of the homogeneous steady states. So when the first mode is set up with a single node in the centre, the first selector gene is turned on in one half of the line of cells and off in the other half. When the second mode is established there are two nodes which bisect the two regions defined by the first switch. If the first modes are of the respective form \backslash and \cup , then the states of gene activity from left to right will be 11, 10, 00, 01. As each new mode appears, its switch will bisect previous regions in such a way that each new compartment is defined by a unique combination of states of gene activity.

This is the essence of the *Drosophila* model. The patterns are more complicated because they are formed not in a line of cells but on various two-dimensional domains: a growing ellipse

represents the growing imaginal disks and an ellipsoidal surface represents the early embryo. It is shown that the nodal lines which appear on an expanding ellipse are very similar to the arrangement of observed compartment boundaries on the fate map of an imaginal disk. The early embryo does not grow in size, but here a fall in diffusion constants due to cellularisation could have an analogous role. It is proposed that five sets of boundaries arise and divide the embryo into 32 regions. The binary coding of the selector genes in these regions corresponds very closely to that predicted from relative transdetermination frequencies, and to the occurrence of certain homeotic mutations.

Is it correct?

If the model were even approximately correct, it would certainly go down in history as a great scientific advance. Its beauty derives from the diverse strands of thinking which come together in the one great synthesis: gradients, thresholds, a binary combinatorial epigenetic code, dissipative structures.

But is it right? In its favour are several impressive retrodictions of known facts. For example, it is known that the anteroposterior subdivision of the embryo commences before the dorsoventral. So the model can lay down both axes of the body with the one mechanism, and the sequence arises naturally from the fact that the long axis of an ellipsoid is longer than

the short axis. Another striking example is the mutant *antennapedia* which converts the antenna into the second leg. The model explains that it must be the second leg rather than the first or third because of their different specific codings.

There are also several known facts which seem unfavourable for the model at the moment. It would be churlish to run through a long catalogue of counter arguments, but one group of phenomena is so strongly at variance that it cannot be ignored. This is the evidence for some sort of monotonic gradient which controls pattern formation in the early embryo. The evidence for a single (or double) controlling gradient is discussed in detail by Sander (*Adv. Insect Physiol.* **12**, 152; 1976) and rests largely on the results of constriction experiments on the eggs of a variety of insect species. There is also evidence for the existence of a persistent monotonic gradient in the imaginal disks which enables fragments to regenerate, sometimes across the compartment boundaries (Bryant *Curr. Top. Devl Biol.* **8**, 41; 1974). The observed behaviours in these experiments are hard to explain by the new model. It should also be borne in mind that the evidence for gradients exists in many developmental systems which have been carefully examined (sea urchin, *Hydra*, and the vertebrate limb for example) while the evidence for binary decisions and a combinatorial code, persuasive as it is, is confined to the insects alone. \square

Replication origins

from David Sherratt

A REPLICON is a contiguous segment of DNA constituting a unit of replication. By definition a replicon controls its own replication; determination of the rate of DNA initiation from a unique origin is generally accepted as the point of control. Most information about origins, initiation and subsequent movement of the replication fork has been furnished by simple viral and plasmid replicons. However, because these depend on their hosts for most of the DNA replication machinery, it should be possible to extrapolate to the more complex host chromosomes.

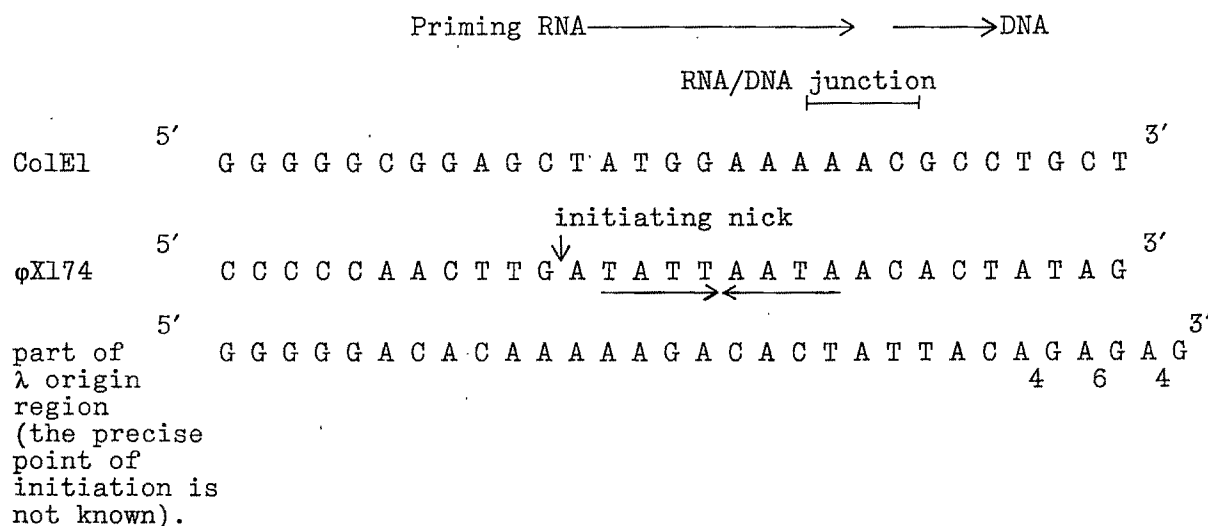
DNA polymerases, unlike RNA polymerases, are unable to initiate polynucleotide chains *de novo*, probably because of the high probability of introducing irrevocable error by mispolymerisation on a short chain, which by

its nature is poorly base-paired to the template strand. Two ways of overcoming this dilemma are known. The most widespread is to utilise RNA 'primers' to provide a base-paired 3'OH group to which deoxyribonucleotides can be added. Since this RNA is 'non-genetic' and is later removed, errors in its polymerisation can be tolerated. Unidirectional or bidirectional replication can follow equally well from such RNA priming. In principle, this type of initiation could be controlled either at the level of initiation of RNA synthesis or at RNA termination/DNA initiation.

Another apparently less common way of initiating DNA replication is to create a deoxyribonucleotide 3'OH primer by nicking a parental DNA strand internally. Replication from such a nick can most simply generate a unidirectional 'rolling circle' and occurs in the replication of the double-stranded DNA bacteriophage ϕ X174.

The new DNA sequencing technology has already been used to determine the sequences at or near both types of origin, including those of phages ϕ X174 and λ , plasmid ColEI and the eukaryotic viruses SV40 and polyoma. F. Blattner's group in Madison has isolated and sequenced a functional λ origin (*Science* **198**, 1041; 1977). Their sequence contains a number of long pyrimidine tracts (one of 18 nucleotides), many runs of greater than four identical nucleotides and a number of direct, inverted and palindromic repeats that allow hairpin loops to be constructed. Unfortunately the functional significance of these features remains obscure, as does the nature of the initiation of λ replication. ColEI vegetative replication is initiated from a RNA primer and Tomizawa and coworkers (*Proc. natn. Acad. Sci. U.S.A.* **74**, 1865; 1977) have sequenced the region about the RNA/DNA junction, which is A-T rich and is preceded one to two helix

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turns before by a G-C rich region. Such a structure is reminiscent of known RNA termination sequences (*Nature* 271, 275; 1978). The same sort of distribution of A-T and G-C rich regions can also be seen in λ and ϕ X174.

In Utrecht, Langeveld *et al.* (page 417 in this issue of *Nature*) have sequenced the region on the 3'OH side of the specific nick in duplex ϕ X174 DNA at which replication is initiated. This nick is introduced by the *cis*-acting ϕ X174 A protein. Reassuringly, this sequence is present in the ϕ X174 total sequence (*Nature* 265, 687; 1977). Adjacent to the nick is a rotationally symmetrical octanucleotide which could be a specific (A-protein?) protein-binding site.

Initiation is likely to be controlled by replicon-specific 'signals' that allow or prevent replication. ϕ X174 and λ each produce initiator proteins (A and O respectively) and it is perhaps not surprising that the genes for these proteins overlap the respective origins of replication. The *E. coli* chromosomal origin is also close to the *dnaA* gene which is involved in replication initiation. In contrast, since ColEI is able to initiate replication in the absence of ColEI-specified proteins, its initiation may be controlled by a repressor.

The ϕ X174 A protein appears to be the prototype of a range of important enzymes which nick DNA but conserve the phosphodiester bond energy by becoming covalently bound to the 5' end of the nicked strand (possibly through a phosphoamide bond, as in the ligase-AMP complex); they are subsequently able to religate the 5' end to the original or possibly a new 3'OH with little or no further energy input. With ϕ X174 this allows a simple mechanism for the production of single strand circular viral strands by a modified 'rolling circle' model (Eisenberg *et al. Proc. natn. Acad. Sci. U.S.A.* 74, 3198; 1977). By analogy, a simple mechanism for conjugal transfer of plasmid DNA and its recircularisation

in the recipient presents itself. Not surprisingly then, ColEI 'relaxation complex', which has similar properties to the ϕ X174 A protein/DNA complex, seems to be involved in conjugal transfer rather than vegetative replication, since work in our laboratory has shown that deletion of the specific site of ColEI relaxation complex nicking abolishes conjugal transfer without noticeably affecting vegetative replication. It would seem that this site is a transfer origin. An SV40 'relaxation complex' that can be nicked close to the origin has also been described (Kasamatsu & Wu *Proc. natn. Acad. Sci. U.S.A.* 73, 1945; 1976).

DNA nicking-closing enzymes also belong to this class. A particularly interesting example is *E. coli* DNA gyrase, which *in vivo* introduces negative superhelical turns into covalently closed DNA. Nalidixic acid, a potent inhibitor

of DNA replication has recently been shown to interact with DNA gyrase. *In vitro*, gyrase can introduce negative superhelicity (in the presence of ATP), remove superhelical turns, and in the presence of nalidixic acid and SDS can cleave seemingly specific phosphodiester bonds, becoming covalently bound to the 5' end of the breaks in the process (Sugino *et al. Proc. natn. Acad. Sci. U.S.A.* 74, 4767; 1977; Gellert *et al. Proc. natn. Acad. Sci. U.S.A.* 74, 4772; 1977). Since introduction or removal of superhelical turns needs continuous nicking-closing activity, the conservation of phosphodiester bond energy is clearly important. DNA gyrase has been clearly implicated in DNA replication, yet its *in vitro* properties also make it, or similar 'break-rejoin' enzymes, candidates for involvement in recombination and transposition. \square

A new type of chemisorptive bond

from R. H. Williams

DURING the past few years there has been great progress in our understanding of atomically clean solid surfaces and of the nature of chemisorption on them. This has been mainly due to the combination of new surface sensitive electron spectroscopic and other techniques (see Woodruff *Phys. Bull.* 27, 165; 1976) with sophisticated theoretical calculations of the surface on an atomic level. These studies are important in understanding processes such as catalysis, corrosion or the behaviour of many electronic devices.

Silicon has been particularly thoroughly studied in this respect because of its great technological importance and the availability of ma-

terial of high perfection. Clean (111), (110) or (100) silicon surfaces contain bonds which are not attached to other atoms, the so called 'dangling bonds'. For the (111) surface there is just one dangling bond per surface atom, extending out normal to the surface. Adsorbed gases such as chlorine bind to this bond, as illustrated in the beautiful recent angularly resolved photoelectron spectroscopic experiments of Larsen, Smith and Farrell (*Proc. V Int. Conf. Vacuum Ultraviolet Rad. Phys. Montpellier*, Extended Abstracts, II, 241; 1977).

On the theoretical front calculations of the surface electronic and vibrational structure have been made by several groups. In particular Appelbaum and Hamann at Bell Telephone Laboratories have conducted a series of

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calculations in which the charge and potential are treated self consistently (see *CRC Crit. Rev. Solid State Sci.*, August, 357; 1976) for a number of plausible clean surface structures and ones covered by adsorbed atoms. Although low energy electron diffraction gives the symmetry of the surface unit mesh the exact positions of the surface atoms are not known precisely. Figure 1a shows the form of the (100) surface, the atoms labelled 1 being the topmost layer, 2, 3, and so on being in subsequent layers. Atoms 1 have two unsaturated bonds. Obviously gases such as atomic hydrogen form strong covalent bonds with these unsaturated electrons. Since all the bonds associated with atom 2, in the second layer are saturated one would not expect hydrogen to form covalent bonds with these atoms.

In a recent paper, however, Appelbaum, Hamann and Tasso (*Phys. Rev. Lett.* **39**, 1487; 1977) conclude that if a hydrogen atom is located directly above atom 2 in Fig. 1 then it can form an unconventional type of covalent bond with the underlying silicon atom. As the hydrogen atom is brought to the surface immediately above atom 2 the charge distribution in the system as well as the force between the atoms is calculated as a function of separation d . It is shown that the hydrogen atoms have little effect on the dangling bond states. For $d = 3.7$ a.u. there is an attractive force between the hydrogen and silicon and as d decreases the attractive force also decreases until $d \sim 2$ a.u., with an accompanying polarisation of the charge around the silicon atoms in the second layer. It then increases rapidly until $d \sim 1$ a.u. and subsequently again decreases, reaching zero for $d \sim 0.25$ a.u. For values of d below 1 a.u. the behaviour of this force relationship is similar to that for hydrogen above a surface silicon atom. In addition the bond charge between the first and second layer silicon atoms is reduced by about 20%, weakening the bond and presumably allowing the bond distance to expand. In fact a simple interpretation of this unconventional covalent bond is that it is made up from valence-bond orbitals centred on the bond charge between the first and second layer Si atoms, and the hydrogen 1s state, somewhat analogous to the multicentred bonds formed in some boron compounds. The bonding combination leads to a tightly bound state, the anti-bonding combination yields a partially occupied state in the Si band gap, whilst the non-bonding combination weakens the Si-Si bonds. In addition the polarisation surface state noted for large d drops to an energy ~ 1.5 eV lower than for the conventionally bonded hydrogen on Si (111). It seems very likely that this

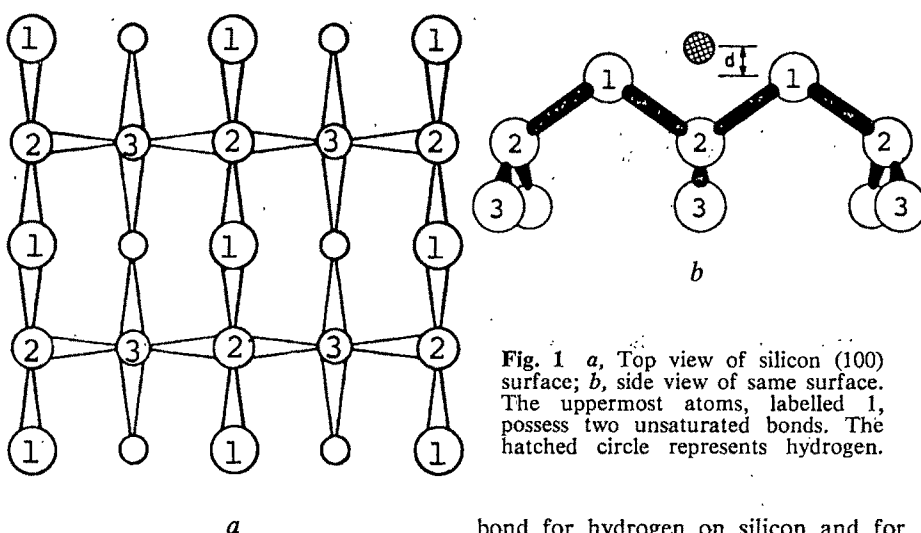


Fig. 1 a, Top view of silicon (100) surface; b, side view of same surface. The uppermost atoms, labelled 1, possess two unsaturated bonds. The hatched circle represents hydrogen.

state has been detected experimentally in the photoemission experiment of Sakurai and Hagstrum (*J. Vac. Sci. Tech.* **13**, 807; 1976) when hydrogen was adsorbed on Si (100) at room temperature.

Undoubtedly there will be further experimental and theoretical investigations of this new type of chemisorptive

bond for hydrogen on silicon and for other systems. It is of interest to note that such bonds may be of importance in amorphous silicon films prepared from silane by the R.F. glow discharge method and first prepared in n and p type form by Spear and LeComber (*Phil. Mag.* **33**, 935; 1976) at the University of Dundee. This material incorporates hydrogen in the lattice and promises to be of considerable use for manufacturing cheap solar cells. □

A new generation of elementary particles

from F. E. Close

EACH year the British high energy physics community meets at the Rutherford Laboratory to review the status of the field. In the past 3 years the discoveries of charm and the unification of two of the natural forces (electromagnetism and the weak force of radioactivity) have been highlights of the meetings. This year's meeting was perhaps even more exciting as discoveries in the past few months have heralded the opening of a new chapter in our conception of the microworld.

Three generations of elementary particles

Our everyday world can be loosely thought of as built from a hydrogen atom template (an electron and proton system) with neutrons (forming nuclear isotopes) and the radioactive phenomenon where a neutron transmutes into the proton with emission of an electron and also a ghostly neutrino (ν_e). The electron and neutrino are a pair of elementary particles called leptons. The proton and neutron on the other hand are now believed to be clusters of elementary particles called quarks. The quarks in the proton and neutron are of two varieties (or flavours), 'up' (u) and 'down' (d). Particles like the proton are made up of three quarks—two ups and one down for the proton itself—whereas

the other class of particles called mesons, that act as the carriers of the strong force between protons and neutrons, contain a quark and an antiquark. The best known meson is the pion (π) which is a combination of an up and a down quark but there are many other mesons, such as the heavier ρ meson and the much-disputed A_1 meson.

This pair of up and down quarks has many properties in common with the lepton pair and there appears to be a deep connection between them. These lepton and quark pairs are today known as the 'first generation' of elementary fermions.

For a reason not yet understood Nature repeated itself. The muon (seemingly a heavy version of the electron (see *News and Views* **266**, 679; 1977)) was discovered over a quarter of a century ago. It too is partnered by a neutrino and hence there is a 'second generation' of leptons (μ , ν_μ). We now know that there is also a second generation of quarks. The strange particles contain a strange (s) quark. The discovery of the J/ψ in 1974 was the first evidence for a charmed quark (c). In 1976 it was found that the c and s quarks are indeed brothers, dramatically in accord with theoretical predictions (*Nature* **262**, 537; 1976).

Charm, the sensation of the past 3 years, took a back seat this year. Interesting discoveries were reported which

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further support the charm hypothesis (see also *News and Views* 269, 286; 1977) and these are adding greatly to our understanding of quark dynamics. While much exciting work still lies ahead, charm is now 'in the textbooks', and the discovery of the decade was overshadowed this year by the astonishing discovery that Nature seems to employ a 'third generation' of elementary particles.

A new heavy lepton — τ

For 2 years there have been signs that a new heavy lepton τ exists. Although the consensus of opinion supported this view there was always some doubt as to whether the signals that were being attributed to the τ (whose mass was around 1,800–1,900 MeV) were really arising from decays of the lightest charmed particles (1,865 MeV).

A very important result seems to be emerging from DORIS, the e^-e^+ colliding beam facility at Hamburg. Since the lightest charmed particle is at 1,865 MeV then 3,730 MeV of energy is needed to produce pairs of charmed particles. Hence the particle ψ' (3,684 MeV) cannot decay into pairs of charmed particles and yet the signals that seem to characterise the production of pairs of the hypothetical τ lepton are being seen in decays of ψ' (3,684). Hence the signals are genuinely from τ , and not charm. Moreover the mass of τ is now believed to be within 20 MeV of 1,810 MeV, some 50 MeV lighter than the lowest charm mass.

Decay properties have been studied and are in line with what would be expected if the τ is accompanied by a new neutrino ν_τ (analogous to the ν_e and ν_μ , first and second generations). There is however a tantalising puzzle: the τ is seen to produce the ρ in its decays in accord with calculation; also the A_1 is seen (confirmation of the A_1 is an interesting 'old' physics by-product of this new world); however, no sign of the π is seen. This should be produced in $\tau \rightarrow \pi + \nu_\tau$. One suggestion put forward is that the ν_τ has spin 3/2 (unlike spin 1/2 for ν_e , ν_μ). If so, a whole new ball game lies ahead.

Another possibility is that the experimentalists should look harder.

A fifth quark?

If there is a third generation of leptons, theorists have argued that there should be a third generation of quarks. This new quark pair is labelled t and b for 'top' and 'bottom'.

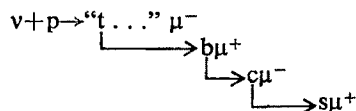
In the summer of 1977 a massive Y (upsilon) particle (mass 9.4 GeV—three times that of the ψ , and nine times heavier than the proton) was seen in muon pairs produced by proton–proton collisions at Fermilab. It is now confirmed that there is also an Y' some 600 MeV heavier than Y . This is tantalisingly the same mass differences as in $\psi' - \psi$ and so theorists speculate that these Y are bound states of a new quark (bottom seems to fit

better with the production rates than does top), and that the quarks feel a potential that rises logarithmically with the inter-quark separation (since this leads to splittings in mass for $\psi'\psi$ or $Y'Y$ independent of the mass scale).

Confirmation of the Y world has come from the intersecting storage ring at CERN where an enhanced production of lepton pairs is seen in the 9–10 GeV region—their resolution cannot separate Y and Y' however.

Bottomless particles?

The ψ had hidden charm—formed by a charmed quark and a charmed antiquark binding together producing a particle with net charm zero—and particles with naked charm were subsequently found. Hence, if the Y has 'hidden bottom' (made from bottom quark and its antiquark) then one predicts that 'naked bottom' particles will exist. Similarly if top quarks exist then 'naked top'—or 'topless' states will eventually be found. The prudish may care to note that t and b are said to stand for truth and beauty, rather than top and bottom, by some physicists. It is predicted that these will decay in a cascade as follows: top decays weakly to bottom, bottom to charm and charm to strange. At each stage a lepton can be emitted. Hence one might anticipate that when a neutrino interacts with a proton several leptons may be produced, for example



A beautiful event with four energetic muons was reported at CERN last month. It is still too soon to say whether this is the first clue in the search for topless and bottomless particles or whether it is a manifestation of something 'conventional' like charm.

Where now?

The phenomenology of bottom quarks was described by J. Ellis (CERN). In particular he drew attention to work of Kobayashi and Maskawa in Japan (in 1972) which showed that the mysterious phenomenon of CP violation emerges rather naturally in a world with six quarks. If the third generation of quarks is indeed filled by subsequent discovery of the top quark then this phenomenon may at last be understood. The search for 'top' is now on.

H. Fritsch (CERN) led a discussion of the impact of the new discoveries on attempts to build gauge field theories unifying weak, electromagnetic and strong interactions. The latter part of this idea is exploitation of the theory of quantum chromodynamics (QCD) which has been developed over the past few years as a quantum field theory of strong interactions which describes the interactions

between quarks. G. Parisi (Ecole Normale, Paris) described some of the phenomena that QCD predicts should be seen in highly inelastic lepton scattering on protons and nuclei. T. Quirk (University of Oxford) showed data from such experiments which appear to be in accord with QCD predictions.

H. Lipkin (Weizmann Institute, Israel) described some exotic multi-quark states that should exist even with the first generation of quarks (see *News and Views* 266, 201; 1977) and discussed evidence for 'exotic nuclei' (dibaryon resonances) that may fit in with these ideas.

The evidence that Nature exploits gauge theories has led to an upsurge of interest in them and E. Corrigan (University of Durham) described some of the rich and exciting structure that is emerging from these studies. Theorists are preparing themselves for the possibility that we are now *en route* for a grand synthesis of all natural forces, including gravity. G. Gibbons (University of Cambridge) described recent work in quantum gravity. Physicists already punch drunk with new quarks, new leptons, monopoles and merons, were now introduced to gravitational monopoles, nut and bolt solutions and even had to contemplate a foam of black holes. When all of these incredible discoveries are finally welded together a profound conception of Nature seems likely to emerge that will make Star Wars seem child's play. □

Homo or hetero?

from Robert W. Cahn

At first sight, an ordered metallic solid solution is as simple and perfect as a crystal can be. Solvent atoms sit on one kind of lattice site, solute atoms on another. The supposed simplicity and perfection are deceptive, for any ordered alloy differs from a plain inter-metallic compound in that its ordered structure is unstable and dissipates on heating before the solid melts. The interest of such alloys does not lie in the perfection of the order, but in the mysterious intermediate states in which order begins to disappear; the halfway house between perfect crystallographic order and an utterly random distribution of atoms on the available lattice site. Are the wrongly sited atoms scattered at random among the 'right' ones—is there a randomness within the randomness—or are there minute groups of 'wrong' atoms, right with respect to their immediate neighbours but wrong with respect to the main mass of the ordered crystal? The first is a homo-

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geneous model of partial order, the second, a heterogeneous one. The homo/hetero dispute goes deep in this field of solid state physics.

When an ordered alloy is heated above its critical temperature, all long-range order disappears, but local or short-range order persists. Its persistence may be deduced from the observation of diffuse X-ray scattering in place of sharp superlattice lines, or from small thermal, electrical or dilatometric anomalies. The atomic format of short-range order is subject to the same uncertainty between homogeneous or heterogeneous models. One view is that a short-range-ordered alloy is homogeneous in the sense that any solvent atom has statistically the same likelihood of having like or unlike nearest neighbours as any other solvent atom. Of the several heterogeneous models, the most intriguing postulates a dispersion of tiny fully ordered domains surrounded by a wholly random matrix with a thin boundary in which there is a gradient of the degree of order. In other words, ordered 'precipitates' exist in a disordered matrix. This model, associated with H. P. Aubauer, has occasioned a great deal of controversy since it was first advanced 6 years ago. The difficulty is this: in the normal way, any dispersion of precipitates in a matrix is unstable; the Matthew Principle operates and large precipitates grow at the expense of the small. The same happens with soap bubbles or with raindrops in a cloud. Aubauer (*Acta Met.* 20, 165, 173; 1972) pointed out that a short-range-ordered, heterogeneous alloy remains indefinitely in a state of fine dispersion: the ordered microdomains do not grow however long they are heated. Aubauer attributes this to the presence of an elastic strain gradient around each domain (because the volume per atom is different in ordered and random regions), and the elastic energy is shown to inhibit the coarsening of domains. Aubauer's calculations have repeatedly been challenged on thermodynamic grounds (the story of the dispute is neatly anatomised by J. W. Martin and R. D. Doherty on pages 205-209 of their book, *Stability of Microstructure in Metallic Systems* (Cambridge University Press, 1976).

The homogeneous/heterogeneous dichotomy with regard to short-range order seems to be incapable of final resolution by X-ray diffraction, electron diffraction, electron microscopy or electrical resistivity. Copper-rich, alpha-phase Cu/Al solid solutions in particular have been examined by all these techniques, over a period of 20 years, and though the partisans of heterogeneity have the edge, the arguments have been impossible to resolve decisively.

A deceptively simple investigation has now, to all appearances, tilted the balance firmly in favour of a heterogeneous model (although that still leaves open the question whether Aubauer's particular version is the most apposite). L. Trieb and G. Veith (*Acta Met.* 26, 185; 1978), two Viennese physicists working in consultation with Aubauer, have studied several Cu/Al alloys exclusively by measurements of electrical resistivity. In the past (for instance in a classic study by Wechsler and Kernohan, *Acta Met.* 7, 599; 1959) this has been done by quenching from high temperatures, which leads to great complexities arising from quenched-in vacancy populations which lead to kinetic anomalies. Trieb and Veith avoided large temperature changes: an ultraprecise measurement technique allowed them to cool or heat their samples by only 5 °C, so that in effect the only variable to affect the resistivity was a change in degree of short-range order. By approaching the same end-state from above and below, they were able to define the temperature range in which equilibrium was attainable in reasonable times in their various alloys. They then analysed mathematically the form of the kinetic curves and showed unambiguously that these could only be interpreted on the hypothesis that two different processes were in progress at different rates. Twin-process kinetics cannot be reconciled with a homogeneous end-structure. Trieb and Veith point out that in Aubauer's 'disperse order' model, an alloy of given composition has at each temperature, in equilibrium, a defined volume fraction of ordered domains and a defined mean size of domains. They propose that the equilibrium volume fraction is attained several times faster than is the equilibrium degree of dispersion, following any sudden small change of temperature. The electrical resistivity is sensitive to both variables and can thus be used to follow their changes. For the most concentrated alloy (18 at. % Cu) the disordered matrix virtually disappears and is replaced by antiphase boundaries.

Trieb and Veith have made a very convincing case for the correctness of a heterogeneous model and have shown how powerful a basically very simple technique, allied with extreme precision and careful quantitative analysis, can be.

It is intriguing that the homo/hetero dispute extends also to the structure of oxide glasses. An enormous amount of work has gone into analysis of such glasses by diffuse X-ray scattering, but it is fair to say that the resultant pair-distribution functions cannot reliably discriminate between homogeneous (Zachariasen-type) network models and various models based on non-crystalline

clusters, or clusters of microcrystals of two polymorphic variants in co-existence. This battleground of glass structures still awaits its resolution, which will perhaps come, in due course, from some technique as elegant and simple as that applied by Trieb and Veith to short-range order.

Their study has shown again the limitations of microscopy, however great the resolution and however sophisticated the technique, in resolving details of not-quite-perfect structures. □

Negative deflection angles in heavy ion scattering

from P. E. Hodgson

WHEN two nuclei interact at energies high enough to overcome their mutual electrostatic repulsion their quantum-mechanical wavelengths are usually small compared with their own radii, so that it is possible for many purposes to consider them as classical particles moving along well-defined orbits. If we then think of the scattering angle as a function of the impact parameter we find several curious effects, in particular that several impact parameters can give the same scattering angle. It has now proved possible to distinguish these orbits experimentally.

The orbits can be visualised by considering the interaction for various impact parameters, or distances of closest approach in the absence of all interactions. For large impact parameters the projectile feels only the long-range repulsive Coulomb (or electrostatic) field and is deflected through a small positive angle. As the impact parameter is decreased the nuclei come closer together so the Coulomb interaction is larger and the repulsion and thus the deflection angle is greater. Thus initially the deflection angle increases as the impact parameter decreases.

As the impact parameter is still further decreased the nuclei approach close enough for their short-range attractive nuclear fields to interact. These oppose the repulsive Coulomb force and thus reduce the scattering angle. Eventually as the impact parameter is reduced still further there comes a point when the Coulomb and nuclear fields exactly balance and the particle is not scattered at all. At still smaller impact parameters the scattering angle becomes negative, and the corresponding orbits wind round the

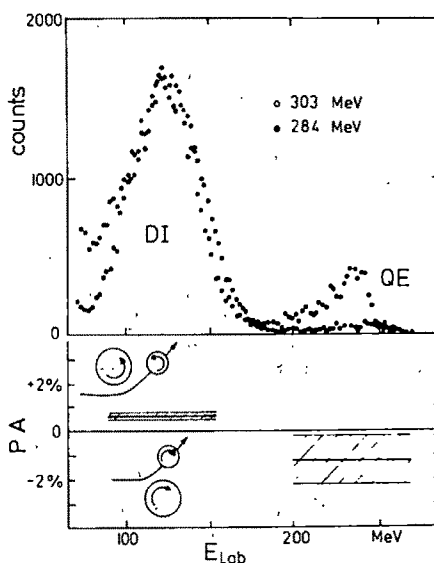
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back of the target nucleus as shown in the inset to the figure.

Although there is a clear physical difference between the orbits corresponding to positive, and negative scattering angles, they cannot be distinguished experimentally just by studying the differential cross sections, since equal number of particles are incident on both sides of the target nuclei. Thus until recently it was not possible to confirm these negative deflection angles experimentally, although they are theoretically very plausible.

It has now been shown by Trautmann and colleagues (*Phys. Rev. Lett.* 39, 1062; 1977) that it is possible to do this by measuring the polarisation of the residual nuclei, which depends on whether the scattering was to positive or negative angles. This is because in these interactions some of the orbital angular momentum of the projectile is given to the target nucleus, so that it is left spinning. If now we consider two orbits that both give emergent particles in the same direction, but one corresponding to positive and the other to negative scattering angle, we find that they leave the residual nucleus spinning in opposite directions, as shown in the inset to the figure. This may easily be seen physically by using the concept of tangential friction; the interactions that take place as the nuclei brush past each other transfer energy and this is felt by the projectile as a tangential force tending to set it into rotation; the same force also sets the target nucleus into rotation.

The particles that have traversed the two types of orbit can be distinguished by the amount of energy they have lost in the interaction. Those scattered through positive deflection angles have been strongly repelled by the Coulomb field and weakly attracted by the nuclear field. Since the loss of energy and the transfer of particles takes place mainly through the nuclear field, such particles will on the average lose rather little energy. On the other hand those particles that are scattered through negative deflection angles have been acted on by a relatively weak Coulomb field but a very strong nuclear field, and so will have lost on the average much more energy. This is illustrated by the figure, which shows the energy spectrum of the particles emitted at 35° from the interaction of 284 and 303 MeV, argon nuclei with a target of natural silver. There are two distinct peaks, the one at higher energies corresponding to the particles that have lost rather little energy in quasi-elastic interactions, and the one at lower energies that have lost much more energy in deep inelastic inter-



The upper half of this figure shows the coincident energy spectra of emerging particles with charges between 11 and 21 from the interaction of 284 and 303 MeV argon nuclei with natural silver. The deep inelastic and quasi-elastic groups are clearly distinguished. The lower part of the figure shows the corresponding polarisations, and the inset shows how these follow from the classical orbit picture of the interaction.

actions.

It is possible therefore to identify the particles that have been scattered through positive and negative deflection angles so all that remains is to show that they have indeed left the residual nuclei spinning in opposite directions. This was done by measuring the circular polarisation of the de-excitation gamma rays corresponding to the two groups. In the initial stages of the de-excitation process charged and neutral particles are emitted, and this will in general reduce the value but not change the sign of the nuclear polarisation. Thus the circular polarisation of the gamma rays emitted in the last stages of the de-excitation process, which has the same sign as the nuclear polarisation, tells us about the direction of spin of the residual nucleus immediately after the interaction.

The results of the measurements of the circular polarisations of the gamma rays in coincidence with the two groups of particles are shown in the figure. Those corresponding to the negative deflection angles have a small positive polarisation, whereas those corresponding to positive deflection angles have a small negative polarisation, which is just what we expect from the theory of the process. This agreement with the theory of the orbits responsible for the positive and negative scattering angles and the energy losses of the particles in the two groups confirms the overall correctness of the picture. This is a remarkable illustration of the value of

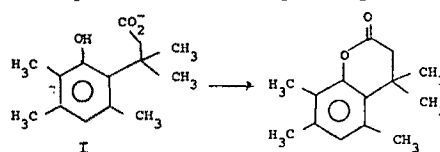
classical arguments in understanding the details of interactions between nuclei at high energies. □

What's special about enzymes?

from G. C. K. Roberts

A Ciba Foundation Symposium on Molecular Interactions and Activity in Proteins was held at the Ciba Foundation on 6-7 December 1977. It was organised by Professor A. R. Battersby, University of Cambridge. The Proceedings will be published by Elsevier/Excerpta Medica/North Holland.

THE symposium was devoted to a discussion of the special catalytic powers of enzymes, and the extent to which this can be explained in precise chemical terms. W. N. Lipscomb (Harvard University) began by summarising the ways in which the remarkable rate enhancements observed with enzymes might be achieved, emphasising that enzymes operate by normal chemical mechanisms, using specific binding to speed things up. He went on to describe model systems which illustrate some of the contributions to enzymic catalysis. Perhaps the most striking example was



the reaction which, due to the 'locked' conformation of compound I is 5×10^{10} times faster than the reaction of the corresponding compound without the methyl groups (Milstein & Cohen *J. Am. Chem. Soc.* 94, 9158; 1972).

It was clear from the examples shown that there are enough factors demonstrable in model systems to account for the power of enzyme catalysis. In which case, what about building a 'model enzyme'? The early stages of an attempt in this direction were reported by J. R. Knowles (Harvard University), who described the design and synthesis of a modified cyclodextrin as a catalyst of phosphate ester hydrolysis. Aromatic molecules are known to bind in the central 'hole' of cyclodextrins, while the catalytic site was provided by three $-NH_3^+$ groups positioned so as to stabilise the transition state. Unfortunately, testing of this ingenious compound as a catalyst was not complete at the time of the meeting; however

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specific binding was demonstrated and it promises to give considerable insight into the requirements for optimal catalysis. Another area in which the organic chemical approach has made enormous contributions to our understanding of enzymes is that of stereochemistry; D. Arigoni (ETH, Zurich) described an elegant series of studies of biological methylation reactions using S-adenosyl methionine labelled with a chiral methyl group (—CHDT).

F. M. Richards (Yale University) described computations of the solvent-accessible surface area of proteins; contrary to earlier generalisations, the surface of a protein is made up of polar and non-polar groups in roughly equal proportions. Richards showed that calculations of changes in surface area can be used to estimate 'hydrophobic' interactions, and that they could form the basis of a program which searched for optimal helix-helix contacts, and which worked well for myoglobin—an encouraging step towards an algorithm for protein folding. He also pointed out that calculations of the packing density of atoms in a protein lead to a 'solid-like' picture of the interior of a protein. This raised the question of flexibility in proteins which was taken up by a number of other speakers throughout the meeting. D. M. Blow (Imperial College, London) discussed crystallographic evidence for large scale flexibility in some proteins, particularly Huber's work on trypsinogen and his own on tyrosyl tRNA synthetase, in which about a third of the subunit fails to give rise to any interpretable electron density. K. Wüthrich (ETH, Zürich) and R. J. P. Williams (University of Oxford) drew attention to NMR evidence for mobility in proteins, principally at the level of single residues. It seems clear that proteins in general are more flexible than the 'solid-like' description implies, although the degree of flexibility varies substantially, not only from one protein to another but within one protein—as exemplified by pancreatic trypsin inhibitor. As pointed out by Wüthrich, this protein is both rigid, in that several peptide NH protons fail to exchange with the solvent on a timescale of years, and flexible, in that the aromatic rings of its tyrosine residues are able to 'flip' through 180° at rates of the order of 10^3 s^{-1} . The possibility that flexibility is in some way essential to catalysis was discussed both by Williams and also by B. Vallee (Harvard University) in describing his studies of arsanilazo-tyrosine 248 carboxypeptidase. Optical absorption, circular dichroism and resonance Raman spectroscopy were used to obtain structural information on the environment of the 'probe', which

could then be related to individual kinetic steps in catalysis.

Turning to substrate binding, C-I. Brändén (Uppsala) described crystallographic work on liver alcohol dehydrogenase, including studies of a ternary complex, enzyme.NAD⁺.p-bromobenzyl alcohol. In this enzyme, the substrate binds at the bottom of a very deep pocket, lined exclusively with hydrophobic residues, where it coordinates to the zinc atom—the only specific enzyme-substrate interaction that is apparent, thus explaining the very broad specificity of the enzyme. Brändén reported that it is very difficult to say from the crystal structures why substrates bind 2–3 orders of magnitude more tightly in the presence of coenzyme; a subtle change in the electronic structure of the zinc may be involved. H. Gutfreund (University of Bristol) described transient-state kinetic studies of the alcohol dehydrogenase reaction, presenting evidence for proton release accompanying the binding of NAD⁺. This proton may well originate from a 'charge relay' system, comprising Asp 293–His 151–Ser 48—(water or alcohol)—zinc, at the active site. An interesting, though as yet unexplained, generalisation noted by Brändén is that in almost all enzymes having a parallel β -sheet structure, the C-terminal part of the sheet contains an anion binding site; in dehydrogenases the pyrophosphate of the coenzyme binds here, in carboxypeptidase the carboxylate of the substrate, and in rhodanese a thiocyanate ion.

Several other speakers took up the question of the specificity of binding in a pharmacological context; G. C. K. Roberts (National Institute for Medical Research, London) discussed the origins of the very tight binding of inhibitors to dihydrofolate reductase, emphasising the role of conformational changes, while M. Halsey (Clinical Research Centre, London) described studies of the binding of general anaesthetics to haemoglobin. T. Blundell (Birkbeck College, London) showed how the combination of the crystal structure with studies of modified insulins makes it possible to define those regions of the molecule which most probably interact with the receptor.

The reactivities and pK_a values of side chains on enzymes can be markedly altered from those of the free amino acids, and this can make a significant contribution to the observed rate enhancement. D. C. Phillips (University of Oxford) and Williams described a combined onslaught, by crystallography and NMR, on lysozyme, asking whether one could predict the reactivity of pK_a of a side chain from its environment as revealed by crystallography. While the exchange rate of the indole NH protons of the

tryptophan residues, for example, correlated well with their solvent accessibility, this was not the case for iodination of the tyrosine residues—the most readily iodinated tyrosine was neither the most solvent accessible nor the one with the most 'normal' pK_a .

Perhaps the most remarkable example of a special environment for a side chain of an enzyme was described by B-M. Sjöberg (Stockholm). Ribonucleotide reductase contains a stable free radical, which is most probably involved in the activity of the enzyme, and which is formed on the side chain of a tyrosine residue, the spin density being located on carbons 1, 3 and 5 of the aromatic ring. The stability of such a radical during purification and handling of the protein is really quite extraordinary.

In his summary, Lipscomb noted that, though we are fairly sure we understand the general principles governing the behaviour of enzymes, we do not know exactly how things work in any individual case. This clearly applies not only to catalytic activity but also to the details of specificity and reactivity. The fact that precise chemical questions can be asked about such systems, if not yet answered, is a mark of the progress that has been made, but theoretical advances will be required before we can properly relate structure and energetics. □



A hundred years ago

ON Monday afternoon a powerful shock of earthquake was felt in the island of Jersey. It was so strong as to cause houses to totter and bells to ring. Its course was from east to west. There was at the time a heavy gale from the south-west in the English Channel. At 11.55 A.M. the same day a shock, lasting about four seconds, was felt at Eastern Alderney. No doubt it was the same earthquake which was felt at Brighton, Blackheath, Fareham, and St. Leonards, as reported in yesterday's *Times*, and at Paris, Havre, and Rouen, as stated by the *Times* Paris correspondent. Mr. Dobson, writing to us from the Royal Victoria Hospital, Netley, Southampton, states that the first shock occurred there at seven minutes to twelve o'clock exactly, and lasted about five or six seconds. It was sufficiently strong to cause the door to shake with some violence, and many objects in the room continued to vibrate for a considerable time.

From *Nature* 17, 2 February, 272; 1878.

review article

Quantification of Earthquakes

Hiroo Kanamori*

Because earthquakes are the result of complex geophysical processes, it is not a simple matter to find a single measure of the size of an earthquake. Further, extremely large earthquakes saturate the conventional magnitude scale. Recent estimates of energy released by such earthquakes suggest values high enough to have possible implications for plate motion, the Chandler wobble and rotation of the Earth.

DURING 1976 a large number of destructive earthquakes occurred: on 4 February, Guatemala suffered an earthquake of magnitude 7.5, with the loss of 23,000 lives; on 6 May, an earthquake of magnitude 6.5 cost Italy 1,000 lives; on 27 July, an earthquake of magnitude 8.0 struck China with the loss of 650,000 lives. The Chinese (Tangshan) earthquake is one of the most disastrous events since the 1556 Chinese earthquake in which 830,000 people perished. Moreover, there have been several other destructive earthquakes in the same year: Fig. 1 clearly demonstrates that 1976 was one of the worst years for earthquake casualties in recent times. However, the damage caused by an earthquake depends not only on its physical size but also on various factors such as when and where it occurs, and Fig. 1 therefore does not necessarily represent the physical 'size' of earthquakes. The question of how we measure the 'size' of an earthquake has been historically a very important yet difficult seismological problem.

Earthquake magnitude scales and seismic energy

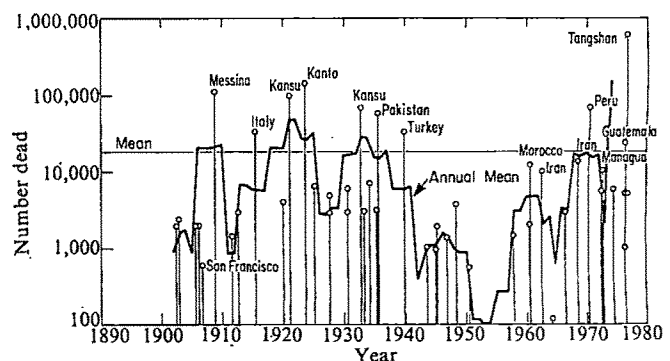
Since the physical process underlying an earthquake is very complex, we cannot express every detail of an earthquake by a single parameter. However, it would be convenient if we could find a single number that represents the overall physical size of an earthquake. This was the very concept of the earthquake magnitude scale, the so-called Richter scale, introduced by Charles F. Richter¹ in 1935. Richter used the maximum amplitude of seismic waves recorded by the Wood-Anderson seismograph. After correcting for the decay of the amplitude with distance from the epicenter, he established the local magnitude scale (M_L) for earthquakes in Southern California, using the logarithm of the observed amplitude of seismic waves. The seismic waves used for local magnitude have periods ranging approximately from 0.1 to 2 s, equivalent to a wavelength of 300 m to 6 km. Since Richter's scale was introduced more or less empirically, it was not quite clear in the beginning what physical parameter of an earthquake this scale represented. However, the Richter scale turned out to be extremely useful for various purposes. The method was so simple that magnitudes could be assigned to hundreds of earthquakes on a routine basis. Since M_L is defined in the period range where the effect of seismic waves on buildings is most pronounced, it is a very good measure of the strength of ground shaking at a given distance, and is very useful for various engineering purposes.

Because of this remarkable success of the M_L scale, Gutenberg² extended this kind of measure to earthquakes in distant sites.

For earthquakes at very large distances, seismic surface waves with a period around 20 s are often dominant on seismograms. Gutenberg therefore defined another magnitude scale, M_S , called the surface-wave magnitude, using the amplitude of surface waves with a period of 20 s. The wavelength of these surface waves is about 60 km. Gutenberg³ also used seismic body waves (P and S waves) to define another scale, m_b , called the body wave magnitude. The period of these body waves is usually from 1 to 10 s. Thus M_S and m_b represent different parts of the frequency spectrum of seismic waves, so that each of these scales represents a different physical parameter of an earthquake. However, extensive studies of Gutenberg and Richter suggested that m_b and M_S seem to be related to each other and could be used to represent a fundamental physical parameter, the energy of seismic waves, E . Through repeated revisions, Gutenberg and Richter⁴ finally derived a relation $\log_{10} E = 1.5 M_S + 11.8$ (E in ergs). This relation made it possible to estimate seismic energy from the magnitude M_S , which can easily be routinely measured. Of course, we cannot expect a very accurate estimate of seismic energy from such a crude parameter through such a simple relation. Nevertheless, this relation provided the only means for quantifying earthquakes in terms of the energy of elastic waves.

The monumental book of Gutenberg and Richter⁵, *Seismicity of the Earth*, catalogues most large earthquakes that occurred from 1904 to 1949. In the later work of Gutenberg⁶ and in the second edition of *Seismicity of the Earth* the catalogue was extended to the period from 1897 to 1952. Fortuitously, after these catalogues were completed, a number of very large earthquakes occurred starting with the Kamchatka earthquake,

Fig. 1 Loss of life caused by major earthquakes. The vertical bars are for the individual event and the solid curve shows the annual average (unlagged 5-year running average).



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$M_s=8\frac{1}{4}$ on 4 November 1952. Here, 'large' refers to the length of the fault. The 1952 Kamchatka earthquake was followed by the Fox (Aleutian) Island earthquake in 1957, ($M_s=8\frac{1}{4}$), the Chilean earthquake in 1960 ($M_s=8.3$), the Kurile Island earthquake in 1963 ($M_s=8.1$), the Alaskan earthquake of 1964 ($M_s=8.4$) and the Rat Island earthquake of 1965 ($M_s=7\frac{1}{4}$). The faults which broke in these earthquakes are extremely long (as long as 800 to 1,000 km for the 1960 Chilean earthquake and the 1957 Fox Island earthquake). No faults that long are known to have ruptured for events before 1952, although the data before 1904 are incomplete. We will use the term 'great earthquakes' for these events with a very long fault.

Seismic waves generated by these great earthquakes are quite spectacular. Very long-period (200–300 s or even longer period) waves which last for several days after the earthquake are often recorded on long-period seismograms. However, the amplitude of relatively short-period (about 20 s) waves generated by these earthquakes is not particularly large, and is about the same as for 'ordinary' large earthquakes. Therefore M_s for these events is not particularly large, in spite of the extremely large fault length and the pronounced long-period waves. Since the energy was calculated only from M_s , the energy release of these 'great' earthquakes was thought to be comparable to that for other 'ordinary' large earthquakes. Why does the amplitude of short-period waves not grow as the length of the fault increases? The amplitude of seismic waves represents the energy released from a volume of crustal rock whose representative dimension is comparable to the wavelength. Several studies⁷ suggest that the energy density per unit volume of crustal rock is almost constant regardless of the size of the earthquake. Since the wavelength of seismic waves used for the determination of M_s is only about 60 km, the amplitude of 20-s waves does not increase as the fault length increases beyond 60 km; therefore the M_s scale saturates. Since these giant earthquakes occurred after the publication of *Seismicity of the Earth*, Gutenberg⁸ and Richter did not have the opportunity to consider the saturation problem.

Not much further effort was devoted to the question of energy estimation until the early 1960s, when remarkable progress was made in long-period seismology. Owing to the worldwide deployment of the standardised long-period seismographs and the development of the theory of the generation and propagation of very long-period (up to 1 h) waves, it became feasible to use very long-period waves for quantitative analyses of an earthquake source. Such long-period waves have wavelengths of several hundreds to several thousands of kilometers, which are comparable to the fault length of great earthquakes, and are therefore suitable for measuring the overall 'size' of great earthquakes.

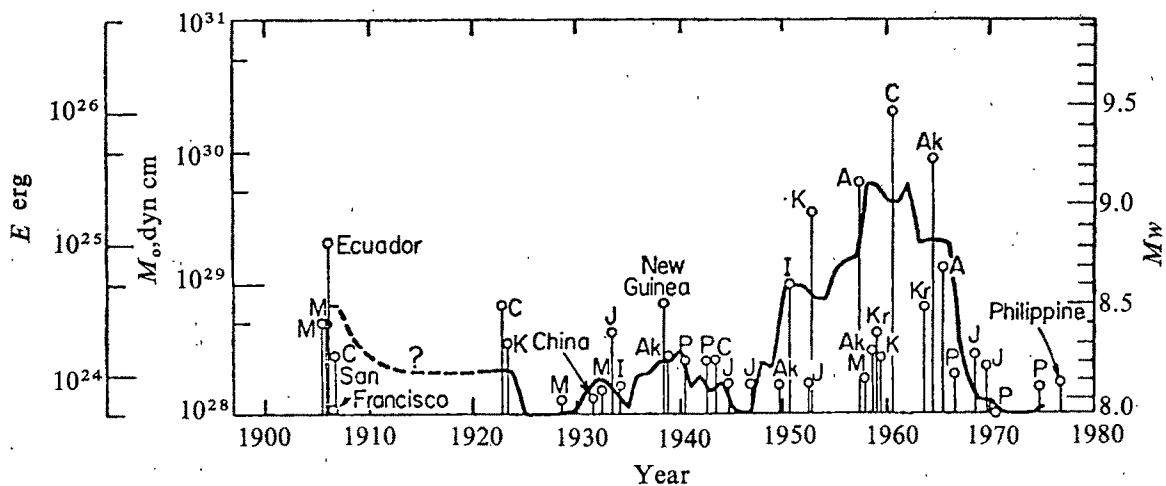
Seismic moment

One of the major breakthroughs in this field was made by the introduction of the concept of seismic moment. Aki⁸ applied elastic dislocation theory to the study of earthquake mechanism. According to elastic dislocation theory, the amplitude of very long-period waves is proportional to the quantity $M_0=\mu DS$ where S is the surface area of the fault, D is the average displacement discontinuity on the fault plane and μ is the rigidity of the material surrounding the fault. M_0 is called the seismic moment. In order to obtain M_0 from the observed seismograms, the effect of propagation, attenuation and the geometry of the fault must be removed. Fortunately, unlike short-period (for example, 20-s) waves, these long-period waves are very coherent so that accurate measurements of M_0 are possible. Thus the seismic moment is one of the most reliable seismological parameters that represents the 'size' of an earthquake. Because M_0 does not saturate, it is a more suitable parameter to represent the size of great earthquakes than the conventional magnitude scales such as M_s .

Several attempts were made to use long-period (100–200 s) waves to quantify large earthquakes^{9–11}. Bruce and Engen¹⁰ introduced a new scale, 100 s magnitude M_{100} , which is based on the amplitude of surface waves with a period of 100 s instead of 20 s. This scale clearly demonstrated that long-period waves are very useful for distinguishing great earthquakes from ordinary large earthquakes.

In recent years, extensive efforts have been made to determine M_0 for great earthquakes as well as large and small earthquakes. The determination of M_0 for recent events can be made relatively easily and very accurately by using high-quality standardised seismograms. The determination of M_0 for old events is less accurate because of the poor quality of old seismograms. Nevertheless, by combining various techniques, the seismic moments of 44 earthquakes out of 52 events of $M_s \geq 8$ which occurred since 1921 have been estimated. For the period from 1904 to 1920, the data are not very complete. Fig. 2 shows the moments for the individual earthquakes as well as the annual average¹². Since the seismic moment directly represents the overall deformation at the earthquake source, Fig. 2 indicates the temporal variation of the amount of crustal deformation associated with earthquakes. It is clear that the seismic activity measured by the seismic moment was very high during the period from 1950 to 1965. The annual average seismic energy release during this period is almost two orders of magnitude larger than that during the period prior to 1950. Is this temporal variation real, or is it an artefact of the poor quality of the record for the period before 1950? Although the quality of the moment determination is somewhat poor before 1950, it is almost certain

Fig. 2 The seismic moment M_0 , seismic energy E , and the magnitude M_w of great and large earthquakes. The solid curve shows the annual average of seismic energy obtained by taking unlagged 5-year running average. A: Aleutian; Ak: Alaska; C: Chile; I: India; J: Japan; K: Kamchatka; Kr: Kurile; M: Mongolia; P: Peru.



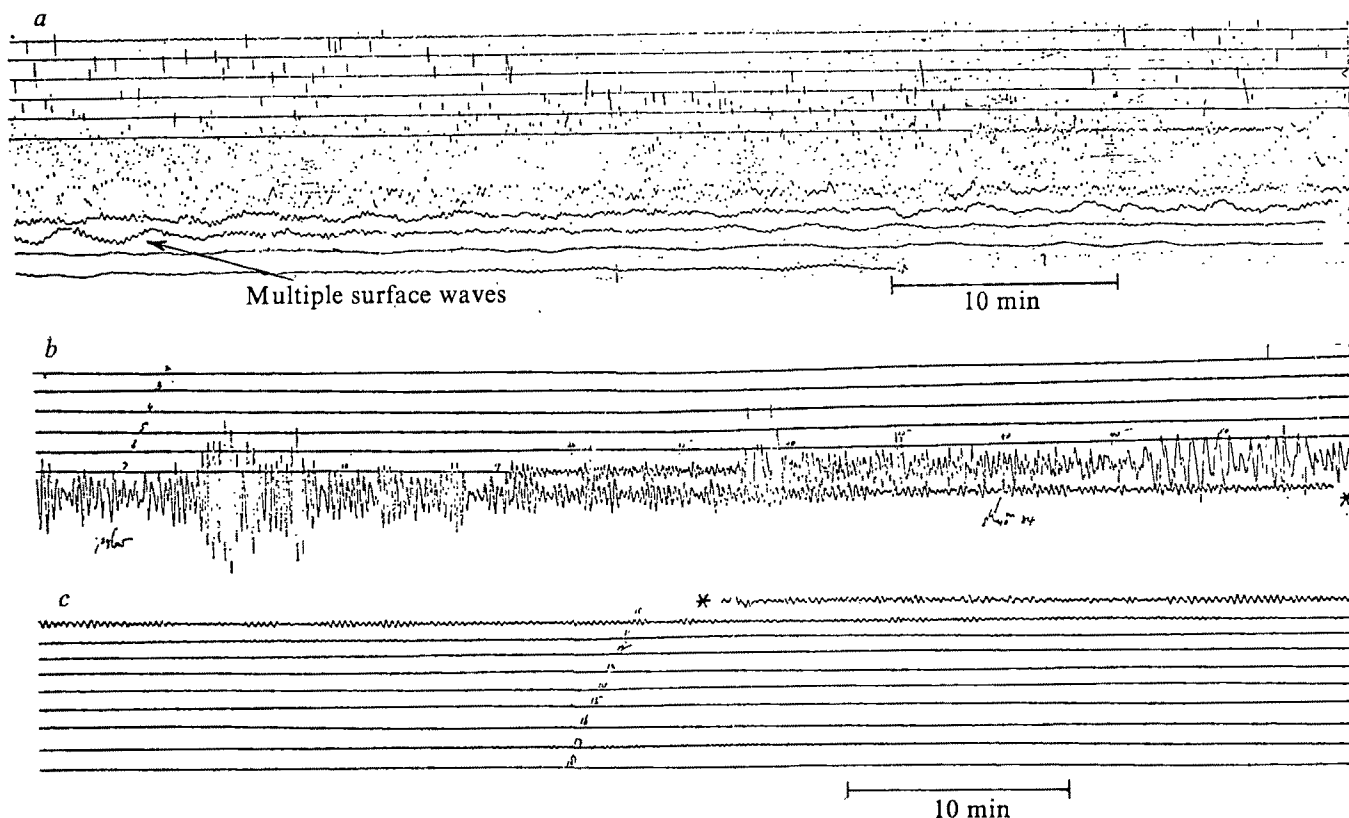


Fig. 3 Comparison of *a*, the 1960 Chilean earthquake ($M_s = 8.3$) and *b*, the 1929 Aleutian Island (Fox Island) earthquake ($M_s = 8.1$) recorded by the Milne-Shaw seismograph. The long-period surface waves—marked as multiple surface waves—which are clearly recorded for the 1960 Chilean earthquake distinguish a great earthquake (Chilean earthquake) from a large earthquake (Fox Island earthquake).

that no earthquake from 1920 to 1950 was as large as the 1960 Chilean earthquake. As shown by Fig. 3, the difference between 'great' and 'large' earthquakes is quite obvious even on old seismograms. Thus, the pattern shown in Fig. 2 is probably real despite the differing quality of the data.

It is also clear in Fig. 2 that the annual average has decreased very dramatically in recent years. This trend is opposite to the trend shown in Fig. 1. This negative correlation clearly suggests that the physical size and the social impact of an earthquake are not directly related.

Seismic moment and seismic energy

The seismic moment can be related to the energy released in earthquakes. Suppose the stress on the fault plane is reduced from σ_0 to σ_1 in an earthquake. Then the reduction in the strain energy is $\Delta W = \sigma D S$ where $\sigma = (\sigma_0 + \sigma_1)/2$ is the average stress, D is the average offset on the fault plane, and S is the area of the fault plane. Combining this with the expression for the seismic moment M_0 we have $\Delta W = (\sigma/\mu)M_0$. If we assume that the fault motion stops when the stress on the fault becomes approximately equal to the frictional stress σ_f , that is $\sigma_1 = \sigma_f$, the above equation reduces to

$$\Delta W - \sigma_f D S = (\Delta\sigma/2\mu)M_0$$

where $\Delta\sigma$ is the stress drop $\sigma_0 - \sigma_1$. Since $\sigma_f D S$ is heat loss during faulting, the left-hand side of this equation gives the total strain energy minus heat loss. Therefore $(\Delta\sigma/2\mu)M_0$ can be considered as the energy available for generation of seismic waves. The stress drop $\Delta\sigma$ is known to be nearly constant for most great and large earthquakes (see for example ref. 13) and $(\Delta\sigma/2\mu)$ is approximately 2×10^{-4} . Therefore $M_0/(2 \times 10^4)$ gives an estimate of seismic energy E . Thus Fig. 2 can be considered as representing the temporal variation of seismic energy release. Note that the energy release in the 1960 Chilean earthquake is 10^{26} erg, which is about 60 times larger than the

value estimated from M_s .

Since the magnitude scale has been used for nearly 40 yr not only among seismologists but also by the news media, it is convenient to express the 'size' of an earthquake in terms of a magnitude scale. To this end, the energy $M_0/(2 \times 10^4)$ can be converted into a magnitude scale by using the energy-magnitude relation, $\log E = 1.5 M + 11.8$. A new magnitude scale, M_w is defined by¹²:

$$M_w = (\log M_0/1.5) - 10.7 \quad (M_0 \text{ in dyn cm})$$

In this scheme, the magnitude is defined in terms of the energy whereas in the old scheme, the energy is calculated from the magnitude. On this scale, $M_w = 9.5$ for the 1960 Chilean earthquake, 9.2 for the 1964 Alaskan earthquake, 9.1 for the 1957 Aleutian Island earthquake, and 9.0 for the 1952 Kamchatka earthquake. In Fig. 2, the M_w scale is given on the right. Comparison of M_w with M_s or M_L shows that M_w agrees very well with M_s and M_L for earthquakes with a smaller fault dimension; thus the M_w scale is a convenient extension of the old magnitude scale to great earthquakes.

Fig. 4 shows the spatial distribution of large earthquakes for the period 1904 to 1976. The values of M_w are given in the bracket for the ten largest earthquakes. It is notable that most of these earthquakes occurred along the Circum-Pacific subduction zones. These ten earthquakes account for more than 90% of the total seismic energy released from 1904 to 1976.

Although this estimate of the energy release of great earthquakes is much larger than that estimated earlier from the magnitude scale, it is still relatively small compared with the average rate of heat flow from the Earth's interior, 7×10^{27} erg yr⁻¹. The average annual rate of the seismic energy release is 4.5×10^{24} erg yr⁻¹, which is about three orders of magnitude smaller than the heat flow. Even for the largest event, the 1960 Chilean earthquake, the seismic energy is 10^{26} erg, which is about 1.4% of annual heat flow. However, the total strain

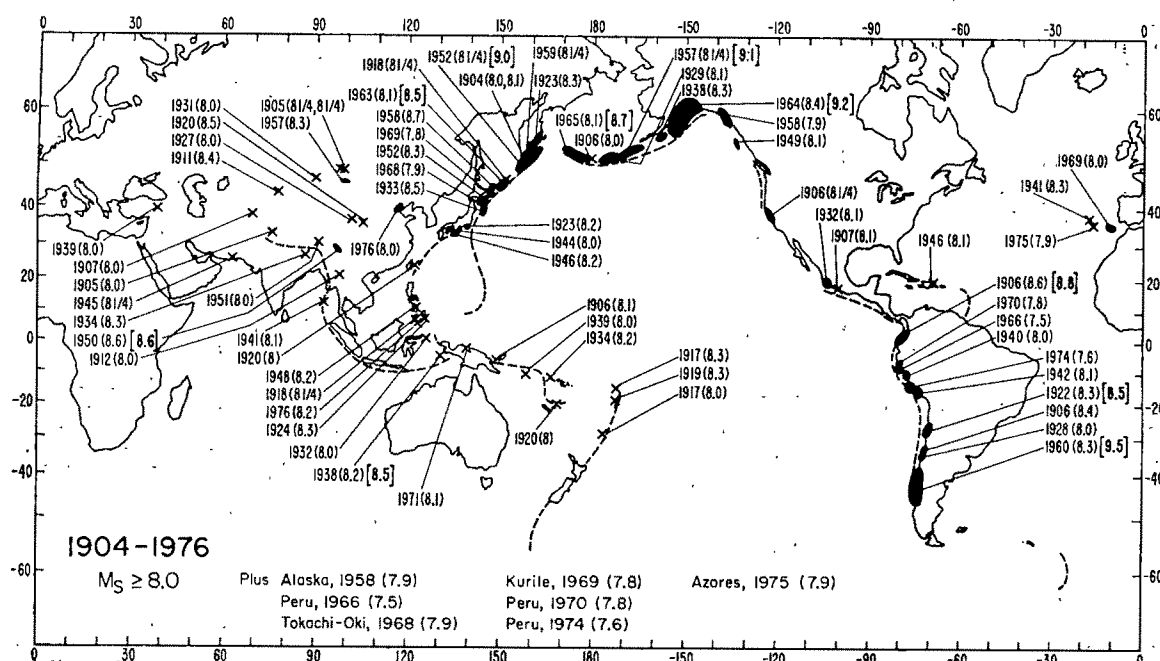


Fig. 4 Great and large earthquakes for the period from 1904 to 1976. The surface-wave magnitude M_s is given in the parentheses and M_w is given in the brackets for the ten largest earthquakes. Major rupture zones are indicated by dark zones.

energy change associated with an earthquake can be much larger (100 times more) than the seismic energy, if the stress drop in earthquakes is only a small fraction of the ambient tectonic stress. The discrepancy between the stress drop in earthquakes (10–100 bar) and the strength of rocks measured in the laboratory (several kilobar) suggests this possibility, but the problem remains unresolved up to now. If this is the case, the total energy involved in the earthquake process can be very significant in the energy budget of the Earth.

Since the total crustal deformation associated with great earthquakes is very large, the study of great earthquakes has a direct bearing on various global problems such as the Chandler wobble, plate motion and the rotation of the Earth^{14–16}. For the moment, the data and analysis are not complete enough to study this problem in detail, but with recent improvements in

instrumentation¹⁷ many important problems in this field may be resolved in the near future.

I thank Robert Geller and Seth Stein for helpful comments.

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articles

Structures and molecular motions in alkaline earth hexammines

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The alkaline earth hexammines have novel structures and molecular motions. The ammonia molecules in these compounds adopt an entirely different geometry from that of normal ammonia. There are two motional transitions as the temperature is increased. The first transition is due to ammonia rotation, and the second results from ammonia diffusion.

STRUCTURE and molecular motion in solids receive a great deal of experimental and theoretical attention and we have initiated a comprehensive research programme to investigate the compounds formed by reaction of elemental metals with ammonia. Only six metal-ammonia compounds are known: $\text{Li}(\text{NH}_3)_4$ and $\text{M}(\text{NH}_3)_6$, where $\text{M} = \text{Ca}, \text{Sr}, \text{Ba}, \text{Eu}, \text{or Yb}$ (ref. 1). All these compounds are metals, and their metallic nature originates from the loss of one, in $\text{Li}(\text{NH}_3)_4$, and two, in $\text{M}(\text{NH}_3)_6$, valence electrons from the elemental metal to a

Table 1 Lattice constants and ^1H NMR parameters

Compound	$a_0(\text{\AA})$	$T_w(\text{K})^*$	$T_s(\text{K})^\dagger$	$E_a(\text{k cal mol}^{-1})$	$\nu_0(\times 10^{-11}\text{Hz})$
$\text{Ca}(\text{NH}_3)_6$	9.01	62	102	3.2	3.3
$\text{Sr}(\text{NH}_3)_6$	9.50	<40	97	2.7	0.5
$\text{Ba}(\text{NH}_3)_6$	9.73	<20	73	2.1	1.0

*Onset temperature for the weak-narrowing transition.

†Onset temperature for the strong-narrowing transition.

conduction band. The motivation for our research is the belief—supported by electrical transport^{2–4}, magnetic^{5,6}, and magnetic resonance⁷ experiments—that these compounds are low-electron-density metals, and as such are rare model systems for testing theories of the electronic behaviour of solids at low electron densities. In addition to the unique electronic properties of metal-ammonia compounds, however, we have discovered that they exhibit both novel structures⁸ and molecular motions⁹. Here we report and interpret the results of our structural and motional studies on the alkaline earth hexammines $\text{M}(\text{NH}_3)_6$, where $\text{M} = \text{Ca}, \text{Sr}, \text{or Ba}$.

The structures of the deuterated compounds have been examined by powder neutron diffraction at 75 K (ref. 8). The sample-preparation technique and neutron spectrometer have been described elsewhere⁸. The hexammines all crystallise in a body-centred-cubic structure. The lattice constants are given in Table 1. To date, only $\text{Ca}(\text{ND}_3)_6$ has been examined in detail, and the atomic parameters were refined by a least-squares fitting of the diffraction profile. $\text{Ca}(\text{ND}_3)_6$ crystallises in the space group $\text{Im}\bar{3}\text{m}$ and has molecular complexes, $\text{Ca}(\text{ND}_3)_6$, located at each lattice site. The six nitrogens in a complex ion are arranged in an exact octahedron around the calcium, with a rather long Ca–N distance of 2.69 Å. The ammonia molecules in $\text{Ca}(\text{ND}_3)_6$ have an entirely different geometry from that of normal ammonia, where the N–H bond angle and distance are 110° and 1.00 Å, respectively. In $\text{Ca}(\text{ND}_3)_6$, the ammonia molecules are nearly planar and have two inequivalent sets of deuterons, with one N–D distance being short (0.94 Å), while the other two are extremely long (1.39 Å). Further evidence for this ammonia structure is provided by the low-temperature proton magnetic resonance (^1H NMR) experiments discussed below. Although each ammonia molecule is coordinated to the calcium by the nitrogen, the pseudotrigonal axis of each ammonia molecule is not coincident with the Ca–N bond, but makes an angle of 13° with it. Furthermore, each ammonia molecule has a 4-fold rotational disorder at 75 K, so that there are a total of 4⁶ possible ammonia orientations in each complex ion. One of the possible structures of the complex is shown in Fig. 1. In addition, there is no hydrogen bonding in the structure and virtually all nonbonded contacts are greater than the van der Waals distances, so that there are essentially no restrictions on the possible ammonia and complex rotations. The temperature factor is 7.4 Å², which indicates considerable thermal motion at 75 K. Although the structures of the other two hexammines have not yet been refined, we anticipate that the hexammines are isomorphous as their unit cells, lattice parameters, ^1H NMR, and chemical behaviour are nearly the same.

Molecular motions

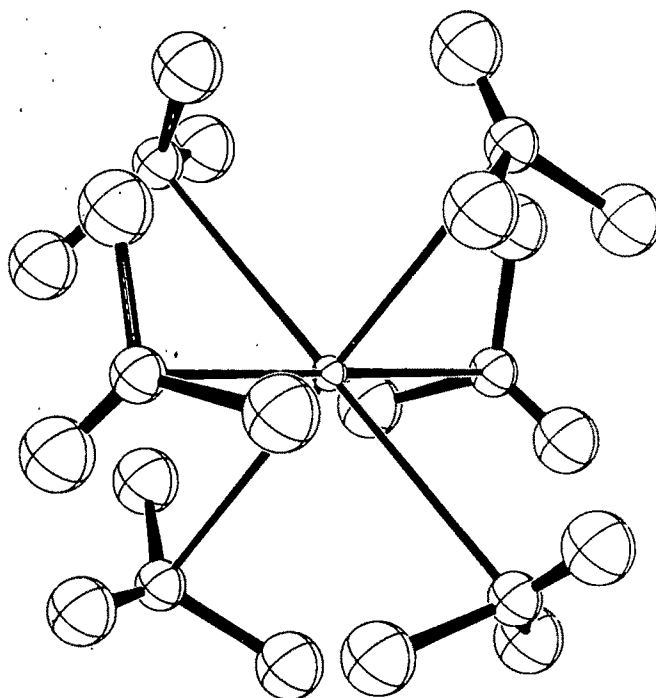
Molecular motion in the alkaline earth hexammines has been investigated by ^1H NMR. The sample-preparation technique and wideline NMR spectrometer have been described elsewhere¹⁰. The spectra consist of a single asymmetrical line, as expected for metals having dimensions much larger than the skin depth, and the asymmetry parameters all lie in the range 1.4–1.9. The temperature dependence of the first-derivative peak-to-peak linewidth, ΔH , is shown in Fig. 2. In $\text{Sr}(\text{NH}_3)_6$, the signal was too weak to detect below 40 K. It is clear from Fig. 2 that all the hexammines exhibit the same qualitative behaviour. In particular, the low-temperature linewidth is about

2 G and, as the temperature is increased, there are two line-narrowing transitions. At the first transition there is a weak narrowing and at the second a very strong narrowing of the ^1H NMR line. The onset temperatures for these transitions are shown in Table 1. The strong-narrowing transitions are completed by 140 K. Above 140 K, the linewidth is independent of temperature and equal to 35 mG. But, because 35 mG is the magnetic-field inhomogeneity on our wide-line spectrometer, high-resolution ^1H NMR experiments were performed to determine the true linewidth. A Varian XL-100-12 NMR spectrometer, equipped with variable-temperature accessory, was used to record high-resolution ^1H NMR spectra in the range 190–300 K. A deuterium lock was employed by placing acetone- d_6 in the outer volume of a coaxial NMR tube (o.d. = 5 mm, i.d. = 3 mm). The measured full-width at half-maximum absorption is independent of temperature in the hexammines and equal to 15 mG. As the hexammines melt near 273 K, the linewidth does not change upon melting. Furthermore, the high-resolution linewidth in liquid ammonia just above the melting point is 11 mG, which is only 4 mG narrower than the hexamine linewidth above 140 K. Below we interpret the ^1H NMR linewidth results in the hexammines in terms of structure and molecular motion.

Linewidth results in hexammines

Using the interproton distances derived from neutron diffraction, the calculated rigid-lattice peak-to-peak linewidth is about 6 G (ref. 11), which is in poor agreement with the low-temperature linewidth of about 2 G. But if, by analogy to solid ammonia¹², the ammonia molecules in the hexammines tunnel at low temperature, then the estimated linewidth is about 3 G (the low-temperature linewidth was estimated by assuming that the intra and inter-ammonia contributions to the second moment are reduced by factors of $\frac{1}{4}$ and $\frac{1}{2}$, respectively, as found for these contributions in solid ammonia at low temperatures¹²), which is in reasonable agreement with experiment. This result supports the neutron-diffraction structure of

Fig. 1 One of the 4⁶ possible structures of the molecular complex $\text{Ca}(\text{ND}_3)_6$ in metallic $\text{Ca}(\text{ND}_3)_6$. The central calcium is octahedrally coordinated to the nitrogens of the six surrounding ammonia molecules.



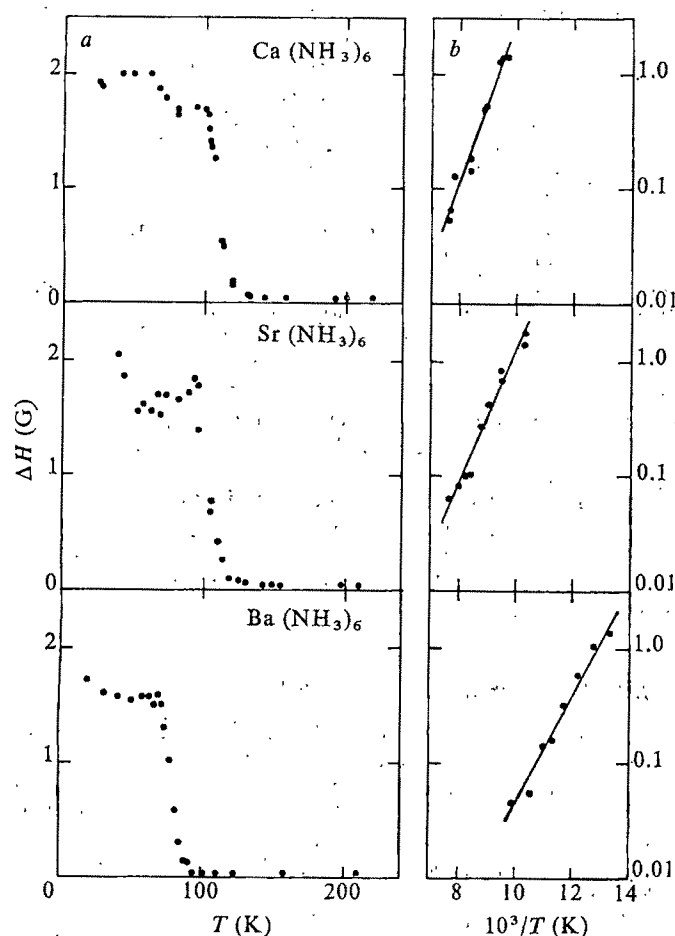


Fig. 2 Temperature dependence of the linewidth (a) and semi-logarithmic plot of linewidth versus reciprocal temperature (b) for the alkaline earth hexammines.

the hexammines as the calculated low-temperature linewidth, using the structural parameters for an ammonia molecule in solid ammonia¹³ and assuming tunnelling, is about 8 G which is a factor of 4 larger than the experimental value.

We attribute the weak-narrowing transition to a transition from quantum-mechanical tunnelling to classical rotation of ammonia molecules. A similar weak-narrowing transition has been observed in solid ammonia between 60 and 80 K, and it was shown to result from the aforementioned rotational transition¹². Further indication of this rotational transition below 77 K in metal-ammonia compounds comes from changes in the electrical resistivity of $\text{Li}(\text{NH}_3)_6$ (ref. 14) and the Mössbauer resonant absorption in $\text{Eu}(\text{NH}_3)_6$ (ref. 15) near 70 K. The decrease in T_w with increasing molecular weight is correlated with a corresponding increase in cell volume (see Table 1); this should lower the barrier to rotation.

The strong-narrowing transition is indicative of other types of proton motion in addition to that associated with ammonia rotation. From the high-resolution ^1H NMR results, it follows that above the strong-narrowing transition the protons diffuse rapidly on the ^1H NMR time scale. Assuming that proton diffusion is a thermally activated process, we used the Gutowsky-Pake equation to analyse the linewidth data for the strong-narrowing transition:

$$\ln \Delta H = E_a/RT + \ln \alpha \gamma (\Delta H_{\text{RL}})^2 / \pi^2 \nu_0$$

where E_a is the activation energy, α is a constant of the order of unity, ΔH_{RL} is the rigid-lattice linewidth, and ν_0 is the vibrational frequency of the jumping atom or molecule¹⁶. (This equation is valid provided that $(\Delta H_{\text{RL}})^2 \gg (\Delta H)^2 \gg (\Delta H_{\text{HT}})^2$, where ΔH_{HT} is the residual high-temperature line-

width. The above inequalities hold over most of the strong-narrowing transition.) The jump frequency is given by $\nu_1 = \nu_0 \exp(-E_a/RT)$, and the reciprocal of the jump frequency is the correlation time, τ_c (ref. 17). As shown in Fig. 1, the semi-logarithmic plot of ΔH against T^{-1} is linear. Taking $\alpha = 1$ and $\Delta H_{\text{RL}} = 6$ G, the calculated values of E_a and ν_0 are summarised in Table 1. Also $\tau_c = 3.5 \times 10^{-8}$ s when $\Delta H = 0.2$ G, and τ_c decreases exponentially with increasing temperature. The low values of E_a and τ_c are consistent with rapid proton diffusion above 140 K. Also, it is interesting that ν_0 is in order-of-magnitude agreement with that expected for the metal-ammonia symmetric-breathing vibration¹⁸.

Mechanisms of proton diffusion

We have considered the following mechanisms of proton diffusion as the most likely possibilities: (1) isotropic reorientation of $\text{M}(\text{NH}_3)_6$ complexes, (2) diffusion of $\text{M}(\text{NH}_3)_6$ complexes, (3) proton exchange alone, and (4) diffusion of NH_3 . We have calculated that mechanism (1) results in a linewidth of about 1 G (noting that the effect of isotropic reorientation is to concentrate the 18 protons of a complex at the metal site, the linewidth was calculated by summing over the four nearest shells of complex ions and then integrating to estimate the remaining contribution. Nearest-neighbour interactions account for 85% of linewidth), so that it could account for only a small fraction of the observed narrowing. Although mechanism (2) would further narrow the line, we consider this process unlikely in view of the large diameter and mass of the $\text{M}(\text{NH}_3)_6$ complex. As T_s and E_a decrease with increasing molecular weight (see Table 1), it supports the contention that mechanism (2) is unimportant. As virtually all nonbonded contacts are greater than the van der Waals distances, mechanism (3) cannot occur by a rotational mechanism. The only other possibility is for (3) to break the N-H bond. However, molecular-orbital calculations have indicated that the N-H bonds in the hexammines are comparable in strength to those in ammonia (INDO calculations indicate that an ammonia molecule in the hexammines is less stable than normal ammonia by about 5 kcal mol⁻¹) so, as in ammonia, proton exchange does not occur until ammonia diffusion sets in.

We conclude, therefore, that the strong-narrowing transition in the hexammines is due to mechanism (4). (Intermolecular proton exchange accompanies proton diffusion in ammonia. It has been estimated, however, that the correlation time for such proton exchange is greater than 10^{-4} s (ref. 19), which is several orders of magnitude larger than the experimental correlation times. Hence the rate of proton exchange is very slow compared to that of ammonia diffusion, so that the integrity of the ammonia molecule is maintained above the strong-narrowing transition.) The ^1H NMR results are all consistent with this. The structural basis for ammonia diffusion is provided by the rather long M-N distance and nonstoichiometry²⁰. The long M-N bond is probably weak and hence can be broken easily, which accounts for the low transition temperatures and activation energies, and nonstoichiometry, in the form of ammonia vacancies, provides available sites to which the ammonia molecules can jump. The strong-narrowing transition may reflect a melting of the ammonia sublattice far below the melting point of the metal, and we believe this would be the first example of molecular-sublattice melting in solid state science. We are investigating this possibility using pulsed-proton NMR and inelastic neutron scattering to elucidate further the details of the motional transitions in the alkaline earth hexammines.

Finally, we shall comment upon the activation energies in Table 1. The experimental activation energies include the M-H bond energy as well as the energy required to transport ammonia, so that they are an upper limit to the N-H bond energy. The decrease in activation energy with increasing molecular weight follows from the corresponding increase in cell volume, which

increases the void volume available for ammonia diffusion and hence lowers the activation energy for transport, and also the expected corresponding decrease in bond energies, which lowers the activation energy for bond breaking.

This work was supported by NSF grant DMR 75-09215 and the Energy Research and Development Administration. We are grateful to the Center for Solid State Science and the Computer Center at Arizona State University for their facilities.

Received 19 September; accepted 23 November 1977.

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Nucleotide sequence of the origin of replication in bacteriophage Φ X174 RF DNA

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The gene A protein of bacteriophage Φ X174 has been used in vitro to convert Φ X RFI DNA into the relaxed RFII form by nicking the viral strand. The nucleotide sequence at the 3' end of the nick has been determined as -- T G C T C C C C C A A C T T G_{OH}. This sequence gives the exact position of the origin of Φ X RF DNA replication.

THE replication of the bacteriophage Φ X174 RF DNA and the way in which the viral gene A is involved in this process has been studied in great detail both *in vivo* and *in vitro*. Results of many workers show that the Φ X174 RF DNA replication starts by nicking the viral strand of the parental RFI DNA which is then elongated from the 3'-OH end under simultaneous displacement of the 5' end, using the circular complementary strand as a template. Therefore the 3'-OH end of the nick is the origin of Φ X RF DNA replication. This rolling circle mode of DNA replication was originally proposed by Gilbert and Dressler¹. The synthesis of a new complementary strand on the displaced viral strand occurs in small DNA fragments and therefore has no unique initiation site^{2,3}.

The location of the origin of replication has been investigated extensively *in vivo*. Repair studies with heteroduplex DNA showed the origin to be within gene A (ref. 4). Using *rep*⁻ cells, the position of the nick in the viral strand of Φ X RFI could be located with high precision in the *Hae*III restriction fragment Z6_B, approximately 185 base pairs away from the Z6_A/Z6_B junction⁵. The termination site for Φ X DNA replication has also been placed in this region of the Φ X genome⁶⁻⁸.

The gene A product of the phage is an absolute requirement for the RF DNA replication *in vivo*⁹. In their studies of Φ X DNA replication in *rep*⁻ cells, Francke and Ray^{10,11} showed that part of the parental RFI molecules was nicked in the viral strand only if a functional gene A product is present. Furthermore the gene A product was shown to be *cis*-acting, that is, in simultaneous infections with phage with an intact gene A and phage with a mutated gene A, only the RF derived from the phage with an intact gene A acquired a discontinuity in the viral strand. This is inconsistent with the asymmetrical

complementation behaviour of gene A mutants⁹. The gene A protein has been isolated since then¹²⁻¹⁴. It is a 55,000 molecular weight (MW) protein and it has a specific endonuclease activity. The A protein nicks Φ X RFI DNA once and in the viral strand only¹² and more precisely within gene A in the *Hind*II fragment R3 (ref. 14). These properties of the gene A protein clearly connect the 3' end of the nick made by the A protein with the 3' end that is the origin of RF DNA replication. The A protein fulfils all the requirements of the initiator protein in the replicon model proposed by Jacob, Brenner and Cuzin¹⁵. It is coded for by the viral replicon, it is required before DNA replication has started and it produces a free 3'-OH end that serves as a primer in the subsequent elongation of the viral strand.

We describe here experiments with the purified A protein and the determination of the nucleotide sequence at the 3' end of the nicked viral strand of RFI DNA that has been incubated with A protein. The sequence found identifies the site of the origin of replication unequivocally in the known nucleotide sequence of Φ X174 (ref. 16).

Gene A protein

THE A protein was isolated from *Escherichia coli* HF4704 (*uvrA*, *thy*, *sup*⁻) cells that had been infected with the lysis-defective phage mutant *am3*. The enzyme purification followed the procedure of Eisenberg *et al.*¹³ with the exception that no chloramphenicol was used during infection. This modification greatly improved the yield of the A protein. The enzyme preparation obtained after DNA-cellulose column chromatography (Fig. 1) contains the two proteins that are coded for by the gene A (ref. 17) (the 55,000 MW A protein and the 37,000 MW A* protein which has no nicking activity¹⁴). This protein mixture was used in the experiments on the endonucleolytic activity of the A protein.

The endonucleolytic activity of the A protein preparations was measured by the conversion of supercoiled covalently closed replicative form DNA (RFI) to the relaxed form containing at least one single-strand nick (RFII). This conversion was visualised on 0.6% agarose gels containing ethidium bromide¹⁸ by the difference in mobilities of the RFI and RFII

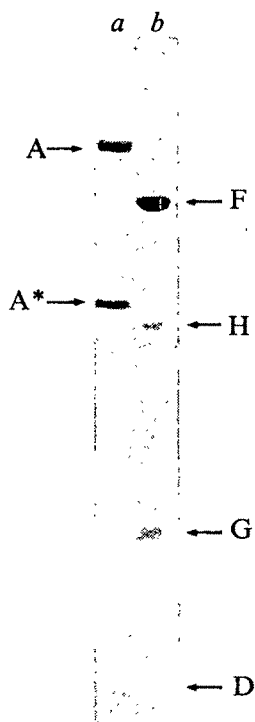


Fig. 1 Sodium dodecyl sulphate (SDS)-polyacrylamide gel electrophoresis of the A protein preparation. The A protein was purified from 15 g *E. coli* HF4704 infected with Φ X174 *am3* for 30 min, following the procedure of Eisenberg *et al.*¹³. Cells were lysed with lysozyme at 37 °C. Solid NaCl was added to a concentration of 1 M and cell debris was removed from the lysate by low speed centrifugation. Ammonium sulphate was added at 0.226 g per ml supernatant over a period of 20 min. After 15 min the precipitate was collected by centrifugation and resuspended in 50 mM imidazole-HCl, pH 6.7, 10 mM β -mercaptoethanol, 1 mM EDTA, 20% glycerol and 1 M NaCl and fractionated on a Biogel A 0.5 m column (150 ml) equilibrated in the same buffer. Proteins eluted from the Biogel column were applied to a Biorex 70 column (15 ml) in buffer containing 0.3 M NaCl. The column was washed with at least four column volumes and finally washed with buffer containing 1 M NaCl. The 1 M NaCl eluate was diluted to 0.4 M NaCl and passed through a single-stranded DNA-cellulose column (2 ml). The A protein was eluted from the column with 1 M NaCl. Each step in the purification was followed by electrophoretic analysis of samples on 12.5% polyacrylamide slab gels prepared according to Laemmli³⁶. *a*, The final A protein preparation after the DNA-cellulose column. *b*, The marker proteins are the phage proteins F, H, G and D with molecular weights of 48,000, 36,000, 22,000 and 14,500 respectively³⁷.

DNA molecules. The amount of RFI DNA converted to RFII DNA was dependent on the amount of A protein but never exceeded 50%. Whether this is the consequence of the presence of the A* protein or not, is unknown. No linear double-stranded DNA (RFIII) was formed, even after incubation with a 10-fold excess of A protein (compared with the minimal amount of A protein needed for 50% conversion). When incubation times were increased to 2 h, in some experiments a minor DNA band was formed that migrated with the mobility of RFI DNA containing no supertwists (relaxed RFI¹⁹). Relaxed RFI DNA formed during incubation of RFI DNA with A protein has also been found by Ikeda *et al.*¹⁴.

Incubation of the reaction mixture with proteinase K before phenol extraction is required for quantitative recovery of newly formed RFII DNA. The strong interaction of the A protein with the DNA causes the extraction of the DNA together with the protein out of the water phase.

The strand specificity of the enzyme activity was tested on RFI DNA labelled with ³H-thymidine in the complementary (—) strand only. Alkaline sucrose gradient analyses of the RFI DNA before and after incubation of this RFI DNA with the A

protein were carried out. The untreated RFI DNA (Fig. 2*a*) sediments much faster than single-stranded DNA. Figure 2*b* shows that after incubation of this RFI DNA with A protein almost all radioactivity in the RFII DNA formed is found after denaturation as circular single-stranded DNA. No increase in label is found at the position of linear single-stranded DNA. This indicates that the A protein nicks only the viral strand of RFI DNA (see also refs 12 and 14).

Site of cleavage

Attempts to label the 3' end of the nick made by the A protein in the viral strand of RFI DNA with terminal transferase²⁰ in the presence of Co²⁺ were unsuccessful. Also treatment of the RFII DNA with bacterial alkaline phosphatase followed by incubation of the DNA with T4 polynucleotide kinase²¹ did not result in incorporation of label at the 5' end. To circumvent the problems with intact RFII DNA, we determined first after digestion with the restriction enzyme *Hae*III in which restriction fragment the nick was located. This nicked fragment was purified and separated into its single-stranded components. The single-stranded viral (+) strand fragments were labelled at their 3' end and the nucleotide sequence at the site of the nick was determined. We were unable to label the 5' end of the nick.

³²P-labelled RFI DNA was digested with the A protein in conditions as described in Fig. 3. The mixture of RFI and RFII DNA was then digested to completion with restriction endonuclease *Hae*III. The digestion mixture was deproteinised by incubation with proteinase K followed by phenol extraction and the DNA fragments were separated on a 5% polyacrylamide slab gel. The separation of the fragments was visualised by autoradiography. The separate DNA bands were excised and the fragments eluted from the gel. All fragments were subsequently electrophoresed on slab gels in conditions in which all secondary structure of the DNA is disrupted²². The two strands of each restriction fragment are not separated in this gel system. Only the double-stranded fragment that contains the nick yields three single-stranded fragments of different size and mobility. This is shown in Fig. 3, the autoradiograph of the electrophoreses in denaturing conditions of the fragments Z6_B, Z7, Z8, Z9 and Z10. Z7, Z8, Z9 and Z10 give a single band but Z6_B is separated into three different fragments, approxi-

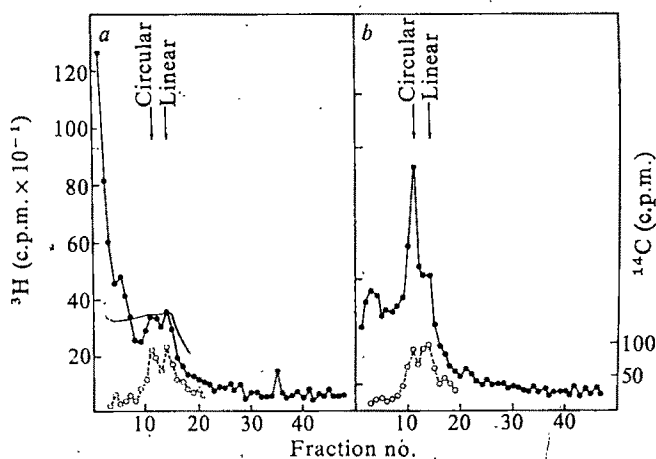


Fig. 2 Alkaline sucrose gradient analysis of RFI DNA treated with A protein. 1.25 μ g RFI ³H-labelled in the complementary strand (●) was incubated with 6 μ g A protein in 50 mM imidazole-HCl at pH 7.0, 60 mM NaCl, 10 mM MgCl₂ and 10 mM β -mercaptoethanol in a volume of 125 μ l at 37 °C for 30 min. Proteinase K (10 μ g), preincubated for 30 min at 37 °C, was added and the incubation was prolonged 20 min at 37 °C followed by extraction with phenol. The DNA samples were layered on 10–30% alkaline sucrose gradients (Baas *et al.*⁵) and centrifuged in the SW41 rotor for 24 h at 38,000 r.p.m. at 15 °C. ¹⁴C-labelled (○) single-stranded linear and circular DNA was added as markers. *a*, RFI DNA without A protein. *b*, RFI DNA after incubation with A protein.

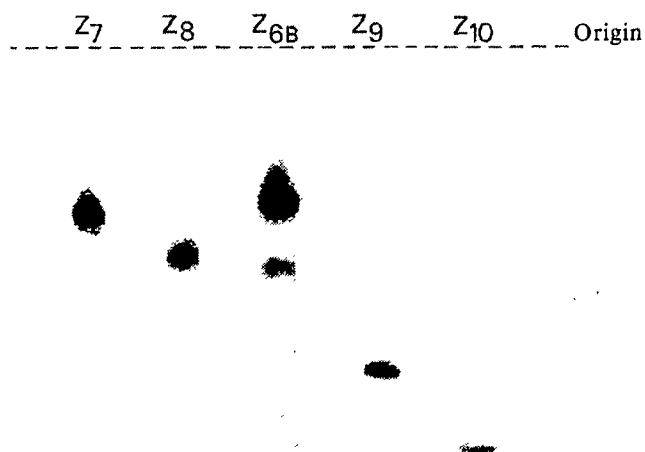


Fig. 3 Autoradiograph of gel electrophoresis in denaturing conditions of the *Hae*III fragments Z_{6B}–Z₁₀. 400 µg RFI DNA was mixed with 50 µg ³²P-labelled RFI (4.6×10^8 c.p.m. Cerenkov) and incubated with 640 µg A protein in the conditions described in Fig. 2 legend. The final volume was 3.2 ml. The DNA was digested with *Hae*III followed by proteinase K treatment and phenol extraction. Fragments were separated on a 5% polyacrylamide gel that had been cross-linked with 0.3% ethylene-diacrylate in 50 mM Tris-borate, 1 mM EDTA pH 8.35, which was also the running buffer. Extraction of the DNA fragments was done by dissolving the gel in 0.5 M ammonium carbonate, 0.1 mM Mg acetate, 0.1% SDS and 100 mM EDTA at pH 9.2 for 16 h at 37 °C. Samples containing the fragments were diluted 20-fold with water and percolated through a hydroxyapatite column. Fragments were eluted with 0.4 M K phosphate, pH 6.8. The recovery was close to 100% for all fragments. The DNA fragments were alcohol-precipitated and dissolved in 20 µl formamide containing 20% sucrose, 0.1% xylene cyanol and 0.1% bromophenol blue and were incubated at 100 °C for 3 min. The longer fragments (Z₁–Z_{6A}) were layered on a 4% polyacrylamide gel made up in 98% formamide (not shown) and the shorter fragments (Z_{6B}–Z₁₀) on a 10% polyacrylamide gel made up in 98% formamide. The gels were made up as described by Maniatis *et al.*²². The length of the fragments in nucleotides is: Z_{6B} 278; Z₇ 234; Z₈ 194; Z₉ 118 and Z₁₀ 78 (ref. 16).

mately 280, 180 and 100 nucleotides in length. The 280 nucleotides band consists of the complementary strand and unnicked viral strand DNA of fragment Z_{6B}. Fragments Z₁ to Z_{6A} give only a single band (data not shown).

From this result we conclude that the nick introduced by the A protein in the viral strand of RFI DNA, is located in the *Hae*III fragment Z_{6B} and cleaves the viral strand of Z_{6B} in pieces of 180 (Z_{6B2}) and 100 (Z_{6B3}) nucleotides each.

The single-stranded fragments Z_{6B2} and Z_{6B3} were eluted from the gel and used for endgroup labelling. Incubation of fragment Z_{6B3} with terminal transferase and α-³²P-UTP in the presence of Co²⁺ in order to label the 3' end²⁰, resulted in incorporation of ³²P label (for details see Fig. 4 legend). The ³²P-labelled Z_{6B3} fragment was partially digested with micrococcal nuclease and spleen phosphodiesterase. The products were separated on the standard two-dimensional system²³ and after autoradiography the pattern presented in Fig. 4 was found. From the mobility shift of the labelled oligonucleotides^{24,25} the sequence of the 3' terminus was determined. The 3' terminal sequence of the fragment Z_{6B3} therefore is -T G C T C C C C C A A C T T G.

The last two nucleotides from the 3' terminus are not part of the *Hae*III recognition site (G G C C) and therefore this 3'

end must have been generated by the A protein. The fact that the 3' end has been extended by terminal transferase, which requires a free 3'-OH, indicates that the terminal G has a free 3'-OH group.

From these experiments we infer that the 5' end at the site of the nick is the same as the 5' end of the fragment Z_{6B2}. Experiments in which we tried to label this 5' end by treating Z_{6B2} with alkaline phosphatase followed by incubation with T4 polynucleotide kinase and γ-³²P-ATP were unsuccessful. This fact together with the observation that the A protein binds tightly to RFI DNA points towards an interaction of the A protein with the 5' end such that this end is no substrate for phosphatase and/or kinase. The presence of a 3'-OH group and a blocked 5' end at the nick has also been suggested by Ikeda *et al.*¹⁴.

Conclusions

The nucleotide sequence found at the 3' end of the endonucleolytic scission made by the gene A protein in the viral strand of RFI DNA has been determined. The nick is located in the *Hae*III fragment Z_{6B} within gene A²⁶. A comparison with the complete sequence of ΦX174 DNA¹⁶ shows that the sequence determined is unique in ΦX174 and corresponds to the nucleo-

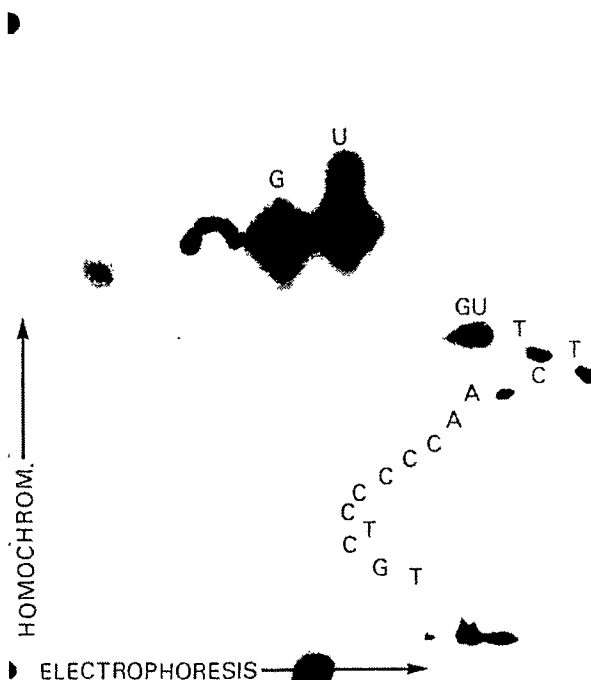


Fig. 4 Autoradiograph of the two-dimensional separation of the oligonucleotides obtained after partial digestion of 3' labelled fragment Z_{6B3}. The DNA fragment Z_{6B3} was eluted from the gel shown in Fig. 3 and labelled with terminal transferase and [α-³²P]UTP in the presence of Co²⁺ by the method of Roychoudhury *et al.*²⁰. Then it was treated with alkali for 16 h at 37 °C, to remove all but the first transferred U residue. When this alkali treated material was incubated with alkaline phosphatase 50% of the ³²P label was removed from the DNA fragment indicating that at least two U residues had been added to each DNA molecule during the terminal transferase reaction. Analysis of a single-stranded DNA fragment with a known 3' end with terminal transferase showed that the terminal transferase was free of exonucleolytic activity. The alkali treated material was alcohol-precipitated, dissolved in 10 mM Tris-HCl pH 9.0, 2.5 mM CaCl₂ and incubated with micrococcal nuclease at 37 °C. After 15 min the pH was lowered to pH 5.0 with HCl and spleen phosphodiesterase was added. The required amounts of micrococcal nuclease and spleen phosphodiesterase and incubation times were determined in pilot experiments. The digestion products were separated in the standard two-dimensional system²³, using electrophoresis on cellulose acetate at pH 3.5 in the first direction and homochromatography on DEAE thin layer using a 3% homomix in the second direction. The oligonucleotides were visualised by autoradiography. Starting from the dinucleotide GU the nucleotide causing the shift of the next longer oligonucleotide is indicated.

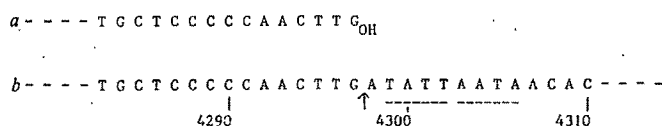


Fig. 5 a, the 3' terminal sequence of Z6_{B3}, deduced from Fig. 4. b, the MRC sequence corresponding to the nick region¹⁶. The A protein cleavage site is indicated by the arrow.

tides 4,283 to 4,297 in the MRC sequence (Fig. 5). The sequence of the 5' end at the nick has not been determined. If we assume that no nucleotides are removed at the nick site, then the viral strand of RFI DNA is cleaved between the nucleotides 4,297 and 4,298. A free hydroxyl group is found at the 3' end thereby making the 3' end a primer for the subsequent elongation of the viral strand. The position of the nick is in full agreement with the *in vivo* origin of replication as determined by Baas *et al.*⁵. Also the C₆T-tract found by Eisenberg *et al.*⁸ near the origin is seen in our sequence 6 nucleotides away from the 3' end. This supports once again the assumption that the 3' end formed by the A protein functions indeed as primer in the synthesis of viral strand DNA and therefore represents the origin for the RF DNA replication.

The nick is located at the start of an A-T rich region (with the palindrome TATTAATA, underlined sequence in Fig. 5b) that is flanked on both sides by a G-C rich sequence (4,282–4,291 and 4,315–4,327 in the MRC sequence¹⁶). Origin regions of SV40²⁷, polyoma virus²⁸ and ColE1²⁹ have been also found to contain A-T rich sequences within G-C rich tracts. The size of and distance between these tracts, however, differ considerably from what is found at the ΦX174 origin. In the phages fd and G4 the region of the origin for the synthesis of the complementary strand on the infecting single-stranded viral DNA is known to contain considerable secondary structure^{30,31}. This is not seen in the ΦX174 origin region but it should be realised that the mechanism of initiation of the complementary strand DNA synthesis is different from the initiation of the viral strand DNA synthesis.

The A protein acts only on RFI DNA containing super-twists. Relaxed RFI is not cleaved³². Supertwisted DNA molecules are known to contain regions of partially single-stranded nature. These destabilised regions are rich in A-T³³. The A protein nicks the viral strand immediately next to an A-T rich sequence (Fig. 5).

If the enzyme needs a specific nucleotide sequence as well as a denatured region in order to nick then this might explain why only supertwisted ΦX174 RFI DNA and not relaxed RFI DNA is a good substrate. After nicking, the RFI DNA formed has a free 3'-OH end and a 5' end that after treatment with alkaline phosphatase cannot be used as a substrate for T4 polynucleotide kinase (neither for DNA polymerase I degradation¹⁴). This suggests a blocked 5' end. This fact together with the observed phenol-resistant binding of the A protein to the RF DNA indicates that the A protein remains attached to the 5' end. This suggests an additional function for the A protein during DNA replication. Covalent attachment of a protein to DNA has been observed with other viruses, for example, adenovirus³⁴ and SV40³⁵.

Received 10 October; accepted 9 November 1977.

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Synthesis and transport of the small subunit of chloroplast ribulose biphosphate carboxylase

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The small subunit of the chloroplast enzyme ribulose biphosphate carboxylase is synthesised as a precursor of higher molecular weight when poly A-mRNA from pea cytoplasmic polysomes is translated by wheat-germ ribosomes. This precursor is taken up into intact isolated chloroplasts, and cleaved to its final size in the absence of protein synthesis. It is deduced that the signal hypothesis does not apply to chloroplasts; an envelope carrier mechanism is proposed.

CHLOROPLASTS contain a distinct genetic system based on a double-helical DNA circle of average length 45 μm, and a 70S

ribosomal protein-synthesising machinery which accounts for up to 50% of the total ribosomal complement of photosynthetic cells¹. The total potential coding capacity of this circular DNA, assuming asymmetric transcription and allowing for an inverted repeat sequence², is about 6 × 10⁶ daltons of polypeptide. It is tempting to conclude that this genetic system synthesises most of the entire spectrum of chloroplast polypeptides, but the evidence does not support this conclusion. Inhibitor studies on intact cells suggest that many chloroplast polypeptides are synthesised by cytoplasmic ribosomes; examples are the Calvin cycle enzymes³ in the case of the stromal compartment, and the chlorophyll *a/b* binding protein^{4,5} in the case of the lamellar compartment. Genetic studies in both algae and higher plants have located the structural genes for several chloroplast poly-

peptides in the nuclear genome^{6,7}. It is thus clear that the formation of chloroplasts requires the integrated activities of both chloroplast and nuclear genomes. Current interest lies in defining the precise contribution of each genetic system and the mechanism of their interaction. Why do chloroplasts contain so many ribosomes, and how do those chloroplast polypeptides made by cytoplasmic ribosomes enter the developing organelle?

Recent technical advances have allowed the isolation of intact chloroplasts which use light energy to drive the synthesis of discrete protein and RNA molecules^{1,8,9}. Analysis of the products of protein synthesis in isolated pea chloroplasts by electrophoresis on one- and two-dimensional polyacrylamide gels has revealed two major products and about 90 minor products¹⁰. One major product is tightly bound to the chloroplast lamellae, and is associated with the ATP-synthase complex¹⁰. The other major product is soluble, and has been identified as the large subunit of ribulose biphosphate carboxylase/oxygenase¹¹, the key enzyme in photosynthesis and photorespiration. This carboxylase (RBPCase) comprises up to 50% of the total soluble protein in leaves, and thus may be the most abundant protein in nature¹². The unusual abundance of this protein could account for the high proportion of ribosomes found inside chloroplasts. The RBPCase molecule is an oligomer of 16 subunits; 8 are termed large (molecular weight (MW) ~55,000) and carry the catalytic sites, while the other 8 are termed small subunits (MW ~14,000). The large subunit is encoded in chloroplast DNA¹³, and is synthesised from a messenger RNA lacking poly A (ref. 14). By contrast, the small subunit is encoded in nuclear DNA¹³; this subunit is not labelled when isolated chloroplasts incorporate labelled amino acids into protein but it has been identified as an *in vitro* translation product of cytoplasmic ribosomes¹⁵. We report here that the small subunit is synthesised as a precursor of higher molecular weight (20,000) when poly A-RNA from pea cytoplasmic ribosomes is translated by a heterologous protein-synthesising system. This precursor enters intact isolated chloroplasts with cleavage to the final size. In direct contrast to other systems where proteins cross membranes^{16,17}, this uptake and cleavage does not require concomitant protein synthesis. We propose that polypeptides enter the chloroplast after synthesis by a mechanism involving combination with specific sites in the chloroplast envelope.

Synthesis of precursor to the small subunit

When seeds of the pea plant (*Pisum sativum*) are germinated in darkness, the etiolated seedlings that result contain small amounts of RBPCase. If such seedlings are exposed to light, this enzyme accumulates rapidly as part of the development of chloroplasts that ensues¹⁸. Polysomes were prepared from etiolated pea apices after exposure to light for various periods by a method which minimises degradation by nucleases¹⁹. Total RNA was extracted from these polysomes and fractionated on a column of oligo dT-cellulose²⁰. The bound fraction containing poly A-RNA was then translated in the presence of ³⁵S-methionine by a protein-synthesising system prepared from wheat-germ²¹. Analysis of the products by one-dimensional electrophoresis on polyacrylamide gels containing sodium dodecyl sulphate (SDS) revealed many labelled bands. One band shows a marked and continuing rise in amount relative to the other bands when the poly A-RNA is isolated from apices exposed to light for progressively longer periods (Fig. 1). This band is specifically precipitated by rabbit antiserum prepared against purified pea RBPCase, but not by preimmune serum (Fig. 2). This immunoprecipitation is competed out by added RBPCase. Since the messenger RNA for the large subunit of RBPCase does not bind to oligo dT-cellulose in the conditions used¹⁴, we conclude that this band contains the small subunit polypeptide. This band has an apparent molecular weight of 20,000—about 6,000 greater than that of the small subunit prepared from purified pea RBPCase. These observations suggest that the small subunit is translated as a higher molecular weight

precursor from a messenger RNA containing poly A; we term this precursor P20. A similar precursor has been reported for the small subunit of RBPCase from *Chlamydomonas*²².

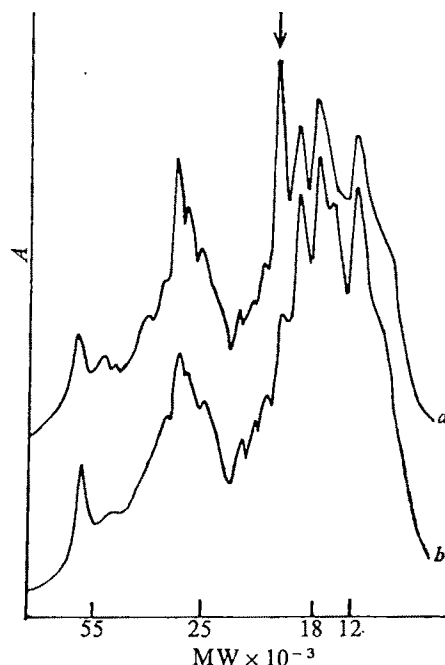


Fig. 1 Products of translation of pea leaf poly A-mRNA. Pea seedlings were grown in the dark for 9 d and then exposed to continuous white light (10,000 lx) for various periods of time. At intervals leaves were frozen in liquid nitrogen and ground to a fine powder. The powder was homogenised in 200 mM sucrose, 200 mM Tris-HCl, 60 mM KCl, 30 mM MgCl₂, pH 8.5 containing 1% (v/v) Nonidet P40 and 0.5% sodium deoxycholate (5 ml for every gram of frozen weight of powder). A polysome pellet was prepared from the homogenate as described previously¹⁹. RNA was extracted from the pellet as described by Brawerman²⁰. Oligo dT-cellulose chromatography was used to obtain poly A-RNA²⁰; LiCl rather than KCl was used because potassium dodecylsulphate comes out of solution readily. The poly A-RNA was used to programme a wheat-germ protein-synthesising system²¹. Each incubation contained in a final volume of 20 µl; 1 mM Tris-ATP, 0.1 mM GTP, 10 mM creatine phosphate, 0.05 mM of each amino acid except methionine, 10 mM dithiothreitol, 110 mM KCl, 2.5 mM Mg acetate, 250 µM spermidine, 50 µM spermine, 8.8 µCi ³⁵S-methionine (800 Ci mmol⁻¹) and 4 µg RNA. The mixture was incubated for 1 h at 27 °C and the trichloroacetic acid (TCA)-insoluble radioactivity determined as follows: aliquots were transferred to strips of Whatman No. 1 paper, heated to 90 °C in 10% TCA containing 0.1% (w/v) unlabelled methionine (10 ml per strip) for 20 min, washed with cold 10% TCA (20 ml per strip), followed by ethanol and ether. The dry strips were counted in 4 ml scintillant (0.5% PPO, 0.03% POPOP in toluene). The remaining assay incubations were prepared for gel electrophoresis by addition of sodium dodecyl sulphate (SDS) and 2-mercaptoethanol to final concentrations of 1% (w/v) and 2% (v/v), respectively, and 1/20 volume of 20×NURB (Neville upper reservoir buffer²⁷). The samples were heated to 95 °C for 2 min and then analysed on slab polyacrylamide gels as described by Laemmli²⁸, using a resolving gel which contained a linear gradient of 7.5–25% (w/v) acrylamide. Electrophoresis was for 16 h at 14 mA at room temperature; the gels were stained in 0.1% (w/v) Coomassie brilliant blue R in 45% methanol, 10% acetic acid and destained in successive changes of 45% methanol, 10% acetic acid. The gels were impregnated with PPO as described²⁹, and the sensitivity of the film increased by pre-flashing³⁰. The film was scanned by a Joyce-Loebl densitometer. The traces are of the products of poly A-RNA isolated from pea leaves greened for a, 24 h; b, 12 h. The arrow marks the band which increases relative to the other bands. Each gel track contains the same amount of TCA-insoluble radioactivity (150,000 c.p.m.); this amount of radioactivity was directed by 0.5 µg added RNA. Controls containing no added RNA incorporated 2,500 c.p.m. The following molecular weight markers were used: RBPCase large subunit (55,000); soybean trypsin inhibitor (25,000); myoglobin (18,000); cytochrome c (12,000).

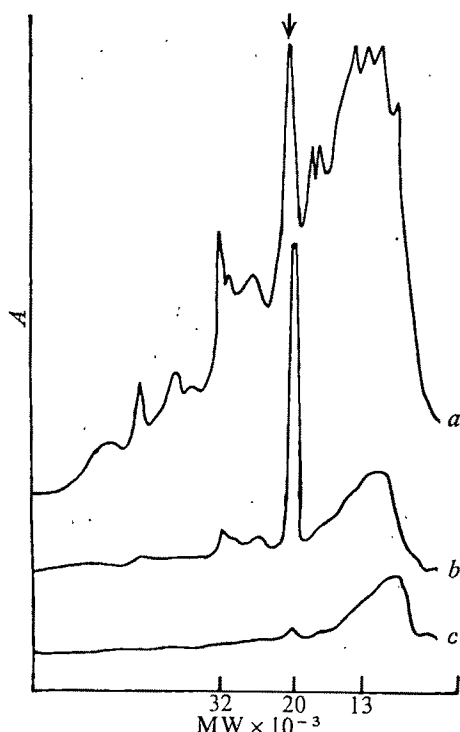


Fig. 2 Identification of small subunit precursor by immunoprecipitation. The ^{35}S -labelled products of translation of poly A-RNA in the wheat-germ system were prepared as described in Fig. 1. After 60 min at 27 °C, the incubations were made up to 100 μl by addition of phosphate-buffered saline (PBS) containing bovine serum albumin (BSA) and Nonidet P40 to final concentrations of 100 $\mu\text{g ml}^{-1}$ and 1% (v/v) respectively. To each incubation 100 μg of rabbit immunoglobulin G (IgG) was added, and the mixture held at 37 °C for 60 min. The specific anti-RBPCase IgG was prepared as described by Shapiro *et al.*³¹, using antigen affinity columns and DEAE/CM-cellulose chromatography; the IgG from pre-immune serum was isolated by ammonium sulphate precipitation followed by the ion-exchange chromatography step. Antigen-antibody complexes were precipitated by addition of excess (10 mg) of heat-inactivated mixture were taken and kept on ice whilst chloroplasts were isolated as described previously¹¹. Chloroplasts from 15 g of 11-d-old pea leaves were washed once in isolation medium and resuspended in 6 ml of 25 mM HEPES-KOH, 110 mM KCl, 3 mM MgCl_2 , 10 mM dithiothreitol, pH 7.6. Aliquots (20 μl) of this suspension were added to the labelled products and incubated at 27 °C for the times stated; controls contained 20 μl resuspension buffer. Chlorophyll was measured by the procedure of Arnon³². *Staphylococcus aureus* (NCTC 8530)³². The *S. aureus* precipitates were washed twice with 250 μl of PBS containing BSA and Nonidet P40. The bound material was released by incubating the *S. aureus* at 50 °C in 200 μl of 10 mM Tris-HCl pH 7.4, 100 mM NaCl, 1 mM EDTA, 1% SDS, 1% 2-mercaptoethanol. The bacteria were removed by centrifugation and the supernatants prepared for polyacrylamide gel electrophoresis and analysed as described in Fig. 1. The tracks are as follows: a, total poly A-RNA-directed products, untreated; b, total poly A-RNA-directed products precipitated by anti-RBPCase IgG and *S. aureus*; c, as (b) but with preimmune serum instead of anti-RBPCase. The arrow indicates the small subunit precursor of apparent MW 20,000.

Processing of P20 to small subunit by intact isolated chloroplasts

Incorporation of ^{35}S -methionine into P20 by wheat-germ ribosomes programmed with pea poly A-RNA ceases after 60 min of incubation at 27 °C. When wheat-germ extract containing labelled P20 is subsequently incubated with preparations containing intact pea chloroplasts, there is a reduction in the amount of labelled P20 and an appearance of label in the small subunit region of the polyacrylamide gel (Fig. 3). The identity of the small subunit labelled by this procedure was confirmed by immunoprecipitation with antiserum to RBPCase (Fig. 4). When analysed by the two-dimensional method of O'Farrell²³, this processing is seen to introduce label into both the electrofocusing variants of the small subunit polypeptide that are found

in *Pisum sativum*. Two observations suggest that this processing step does not require concomitant protein synthesis by either cytoplasmic or chloroplast ribosomes. First, processing occurs after protein synthesis by the wheat-germ extract has ceased, as judged by amino acid incorporation measurements. And second, processing still occurs in the presence of either chloramphenicol (100 $\mu\text{g ml}^{-1}$) or cycloheximide (100 $\mu\text{g ml}^{-1}$). Chloramphenicol is a specific inhibitor of protein synthesis by chloroplast ribosomes, while cycloheximide is a specific inhibitor of protein synthesis by cytoplasmic ribosomes¹. While it is clear that processing does not require concomitant protein synthesis, it is still possible that it requires the presence of ribosomes. This possibility has been ruled out by our finding that processing still occurs even if ribosomes have been removed from the wheat-germ extract by centrifugation (data not shown).

Processing of P20 to small subunit occurs maximally in the presence of chloroplasts which retain their outer envelope (Table 1). Lysed chloroplasts and stromal preparations show some activity (40% and 25%, respectively, of the activity of an equivalent amount of intact chloroplasts). The preparation of lysed chloroplasts is unfractionated and thus still contains fragments of envelopes. The slight processing activity of the stromal fraction can be removed by centrifugation at 30,000 g_{av} (average) for 40 min. Preparations of chloroplast lamellae and heated chloroplasts (2 min at 90 °C) have no processing activity.

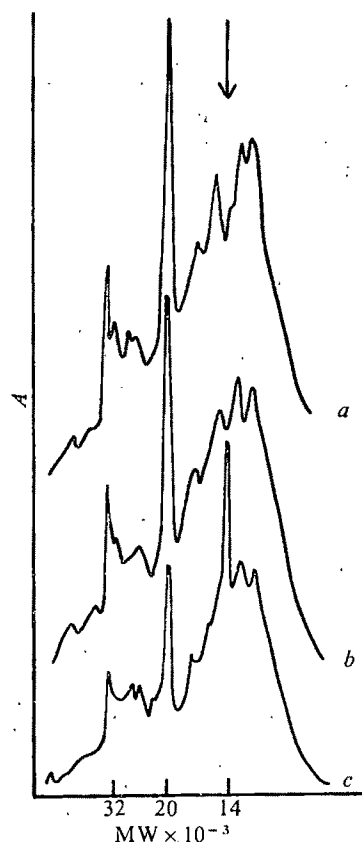


Fig. 3 Processing of P20 to small subunit by intact chloroplasts. Poly A-RNA was translated in the wheat-germ system as described in Fig. 1. After 60 min 10 μl aliquots of the incubation mixture were taken and kept on ice whilst chloroplasts were isolated as described previously¹¹. Chloroplasts from 15 g of 11-d-old pea leaves were washed once in isolation medium and resuspended in 6 ml of 25 mM HEPES-KOH, 110 mM KCl, 3 mM MgCl_2 , 10 mM dithiothreitol, pH 7.6. Aliquots (20 μl) of this suspension were added to the labelled products and incubated at 27 °C for the times stated; controls contained 20 μl resuspension buffer. Chlorophyll was measured by the procedure of Arnon³². Samples were prepared for polyacrylamide gel electrophoresis and analysed as described in Fig. 1. The traces are densitometer scans. Trace a, *in vitro* product incubated with buffer for 60 min; b, *in vitro* product incubated with chloroplasts (5 μg chlorophyll) for 0 min; c, *in vitro* product incubated with chloroplasts for 60 min. The arrow indicates the position of the small subunit.

These observations suggest that the processing enzyme is located in the chloroplast envelope.

The small subunit precursor reported from *Chlamydomonas*²² was also found to be processed to the small subunit, but in this case the processing activity was soluble. It is not possible to isolate intact chloroplasts from this alga; instead, extracts are prepared by the relatively violent method of passage through a French pressure cell. We therefore suggest that the soluble nature of the processing activity in this case may be a result of the procedures used, and not reflect its true location in the chloroplast envelope.

Transport of P20 into isolated chloroplasts

Our conclusion that the processing of P20 to small subunit requires the presence of chloroplast envelopes led us to see whether the small subunit would enter into the intact chloroplast. The commonly accepted criterion for the passage of a polypeptide across a membrane is the appearance of resistance to digestion by an added protease; this resistance should be abolished by the addition of detergents which disrupt membrane structure²⁴. Figure 5 shows the results of experiments designed to apply this criterion to the processing of P20 by intact chloroplasts. The results clearly show that the small subunit produced when labelled P20 is incubated with intact chloroplasts is resistant to hydrolysis by trypsin (track c), unless detergent is added at the same time (track d). Other low molecular weight polypeptides synthesised from poly A-RNA by the wheat-germ extract are all digested by trypsin, even in the absence of detergent. The product with the mobility of small subunit seen in track a was shown not to be authentic small subunit by tryptic peptide analysis (data not shown).

Mechanism for polypeptide transport into chloroplasts

The signal hypothesis provides an attractive mechanism for explaining how polypeptides which are secreted by cells are

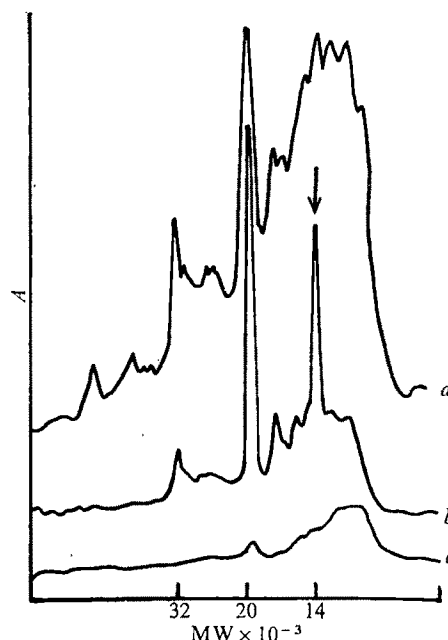


Fig. 4 Identification of product of *in vitro* processing of P20. Poly A-RNA was translated in the wheat-germ system and the labelled polypeptides obtained incubated with intact chloroplasts as described in Fig. 3 except that 10 μ l of wheat-germ incubation mixture was incubated in a final volume of 60 μ l with chloroplasts containing 6.8 μ g chlorophyll. After 60 min at 27 °C the chloroplasts were lysed by addition of Nonidet P40 to 1% (w/v) and the volume made up to 200 μ l with PBS. Aliquots (100 μ l) were analysed by immune precipitation and gel electrophoresis as described in Fig. 2. Electrophoresis was from left to right and autoradiography was for 7 d at -70 °C. The lines are densitometer scans. a, Total poly A-RNA-directed products incubated with intact chloroplasts; b, the polypeptides from a, which bind to anti-RBPCase IgG; c, as (b) but using pre-immune IgG. The arrow marks the position of RBPCase small subunit.

Table 1 Processing of P20 by chloroplast fractions

Incubation for 60 min in the presence of:—	Processing activity
Buffer	0
Intact chloroplasts	100
Lysed chloroplasts	40
Low speed stroma	25
High speed stroma	< 5
Thylakoids	0
Boiled chloroplasts	0

Poly A-RNA was translated in the wheat-germ system as described in Fig. 1. After 60 min, 10 μ l aliquots of the incubations were taken and kept on ice whilst chloroplasts were isolated as described in Fig. 3. Two pellets of chloroplasts were obtained. One was resuspended in 6 ml of 25 mM HEPES-KOH, 110 mM KCl, 3 mM MgCl₂, 10 mM dithiothreitol, pH 7.6 (intact plastid buffer); this preparation is referred to as intact chloroplasts. The other pellet was resuspended in 3 ml of 10 mM HEPES-KOH, 10 mM dithiothreitol, pH 7.6 followed by 3 ml of 2 \times intact plastid buffer; in this preparation all the chloroplasts are lysed. An aliquot (1 ml) of the intact chloroplast suspension was placed, for 5 min, in a boiling waterbath; this gave a boiled chloroplast preparation. An aliquot (4 ml) of the lysed chloroplast preparation was centrifuged at 4,000 g for 5 min, the supernatant was removed and the pellet resuspended in 4 ml of intact plastid buffer. The two fractions are referred to as low speed stroma and thylakoid preparations respectively. Half of the low speed stroma was centrifuged for 40 min at 30,000g_{av}; this gave high speed stromal preparation. Equal volumes of each preparation were added to the labelled products and incubated, in a final volume of 50 μ l, at 27 °C for the times stated. Chlorophyll was measured by the procedure of Arnon²⁵; each incubation contained the equivalent of 4.7 μ g of chlorophyll. Samples were prepared for polyacrylamide gel electrophoresis and analysed as described in Fig. 1. The autoradiographs were scanned on a Joyce-Loebl microdensitometer. The processing activity was determined by comparing the absorbance of the P20 band at 0 and 60 min. Incubation with intact chloroplasts caused a 60% reduction in this absorbance; this decrease was arbitrarily defined as 100 units of activity.

synthesised by membrane-bound ribosomes and transported into the lumen of the endoplasmic reticulum^{16,17}. According to this hypothesis, secreted polypeptides are encoded by messengers which specify an N-terminal sequence of amino acids, whose appearance on the polysome causes the latter to bind to the membrane of the endoplasmic reticulum. The growing polypeptide chain is then inserted through a tunnel in the membrane formed by the association of the signal sequence with membrane proteins; protease activity in the membrane rapidly removes the signal sequence. A key feature of this mechanism is that the transport through the membrane depends upon concomitant protein synthesis by membrane-bound ribosomes, since the polypeptide chain moves through the tunnel in an extended form as it is being lengthened.

Application of this hypothesis to the transport of polypeptides into chloroplasts predicts that the outer chloroplast envelope should be studded with bound ribosomes during the development of the organelle. However, it is apparent from many electron microscopic studies that the chloroplast envelope never presents a similar appearance to rough endoplasmic reticulum. To overcome this objection, a modified signal hypothesis may be considered, in which the chloroplast polypeptides are first inserted into vesicles of endoplasmic reticulum which subsequently fuse with the chloroplasts.

It is clear from the evidence we have presented that the transport of the small subunit of RBPCase into the chloroplast proceeds by a mechanism different from that envisaged by the signal hypothesis. This transport proceeds after synthesis of the precursor has ceased, and in the absence of cytoplasmic ribosomes. Additional evidence consistent with this conclusion is the observation that the small subunit of *Chlamydomonas* is synthesised by free cytoplasmic ribosomes in the absence of membranes²². All these observations are consistent with the

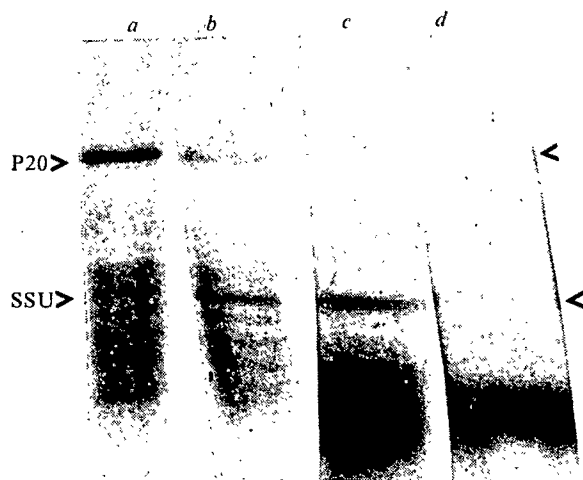
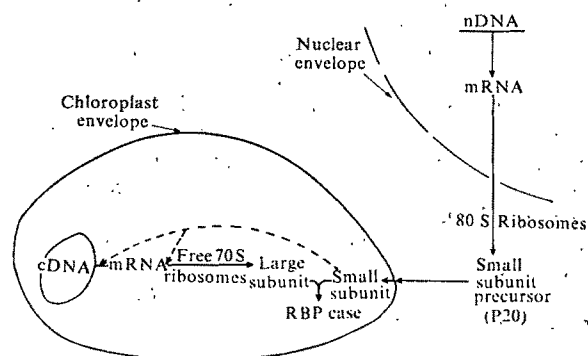


Fig. 5 Transport and processing of P20 by intact chloroplasts. Poly A-RNA was translated in the wheat-germ system and the labelled polypeptides obtained were incubated with intact chloroplasts as described in Fig. 4. At the end of the incubation, TPOCK-treated trypsin was added to a final concentration of $100 \mu\text{g ml}^{-1}$ and the mixture incubated at 30°C for 30 min. To some samples Nonidet P40 (final concentration 1% (v/v) was added at the same time as the trypsin. Samples were analysed as in Fig. 2. Track *a*, polyA-RNA-directed products incubated in buffer without chloroplasts for 60 min followed by a further 15 min in the absence of trypsin; *b*, poly A-RNA-directed products incubated with chloroplasts for 60 min followed by 15 min in the absence of trypsin; *c*, as (*b*), but the final incubation was with trypsin; *d*, as (*c*), but the final incubation was with Nonidet P40 as well as trypsin. P20, small subunit precursor; SSU, small subunit of RBPCase.

envelope carrier hypothesis which was first suggested to account for the transport of polypeptides into chloroplasts when it was demonstrated that isolated chloroplasts synthesise the large but not the small subunit of the RBPCase¹¹. This hypothesis states that a class of proteins exists in the chloroplast envelope which recognises sites common to all those proteins which are made on cytoplasmic ribosomes, but which are destined to function in the chloroplast. In this hypothesis the recognition site on the entering polypeptide need not be at the N-terminus.

The weakness of the envelope carrier hypothesis is that while it accounts for the specificity of transport, it does not propose a mechanism for the actual movement of the polypeptide through the envelope. However, analysis of the processing of P20 on electrofocusing gels shows that P20 has a much lower isoelectric point (by 1.0–1.5 pH units) than either of the two electrofocusing variants of the mature small subunit. This observation suggests that the extra sequence(s) in P20 contain acidic amino acids; known signal sequences, by contrast, are rich in hydrophobic amino acids and contain few, if any, acidic amino acids³⁴.

Fig. 6 Postulated model for RBPCase synthesis in higher plants. nDNA and cDNA stand for nuclear and chloroplast DNA respectively. The dashed line indicates possible control sites at which small subunit affects synthesis of large subunit (modified from ref. 5).



Removal from P20 of such a large number of amino acids (around 50), many of which are charged, is likely to cause a conformational change in the polypeptide. We therefore propose that the transport of P20 into chloroplasts involves combination with a specific carrier in the chloroplast envelope. Protease activity in the envelope then removes part of the polypeptide, which triggers a conformational change leading to the transport of the small subunit across the envelope and release into the stroma.

Aspects of the envelope carrier hypothesis are testable by established techniques. Purified preparations of pea chloroplast envelope can be made²⁵, and will be tested for combination with and processing of P20. The physical state of the small subunit inserted into both intact chloroplasts and purified envelopes will be determined to see whether combination with the large subunit is necessary for the release of the small subunit into the stroma. It is possible that part of the sequence difference between P20 and the small subunit is necessary for the assembly of the RBPCase molecule: this would account for the difficulty in reconstituting active RBPCase molecules from isolated subunits. The suggestion that the small subunit is required for the continuing translation of the messenger for the large subunit⁵ can also now be tested.

Figure 6 summarises our current model for the synthesis of RBPCase in eukaryotic plant cells. The principles exemplified by this model may be of general application to the synthesis of the many proteins found in both chloroplasts and mitochondria. It has been established for each of at least three mitochondrial proteins that their subunits are made in different cellular compartments, for example, three of the seven subunits of cytochrome *c* oxidase are synthesised by mitochondrial ribosomes while the other four are made in the cytoplasm³⁵. The cytoplasmic subunits have been reported to exert a specific and positive effect on the synthesis of the other three subunits by isolated mitochondria in the absence of cytoplasmic protein synthesis³⁶. This finding suggests that in the case of mitochondria as well as chloroplasts, cytoplasmically synthesised polypeptides enter the organelle by an envelope carrier type of mechanism, and control the synthesis of the polypeptides with which they ultimately associate.

Received 10 October; accepted 21 November 1977.

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letters to nature

Cosmic ray source abundance of calcium

MEASUREMENTS of the charge composition of cosmic ray nuclei have led to the belief that cosmic ray calcium nuclei originate in about equal proportions from the sources and from spallation in the interstellar medium¹. The primary calcium is thought to be essentially ⁴⁰Ca, as in the Solar System, while from nuclear reaction systematics the spallative calcium should be mainly ⁴²Ca, ⁴³Ca and ⁴⁴Ca. Simpson *et al.*², however, have presented some surprising results of a measurement of the isotopic composition of calcium in cosmic rays. They found that calcium is largely ⁴²Ca, and they did not see any ⁴⁰Ca. They concluded that calcium is absent at the source, and to account by spallation only for the measured abundances they assumed that the cross-sections for production of Ca by spallation of Fe are much larger than those calculated by Silberberg and Tsao's formula³. A very low calcium abundance in the source would be a major difference with the Solar System, and an experimental determination of the calcium production cross-sections is essential to settle this point. In a report on results of measurements of cross-sections for production of stable nuclides by spallation of Fe (ref. 4) we emphasised the element group Sc-Mn, but some results have also been obtained for Ca, as a byproduct⁵. We considered these as preliminary, and no cross-section value was given for this element. The measurement of Simpson *et al.*² has motivated us to re-examine our calcium data. The cross-sections we

Although the experiment was not specially designed for calcium, this problem was not so crucial here, because of the large difference in isotopic composition between the spallative calcium and the natural one. Contamination is dominant only for ⁴⁰Ca, which represents 97% of natural calcium, but this is not important because the cross-section of ⁴⁰Ca is very small³. On the other hand, isotopes with larger cross-sections have very low natural abundances, so that the contamination, as deduced from the mass-40 peak, is only a few per cent for ⁴²Ca and ⁴³Ca, and at most ~20% for ⁴⁴Ca.

In Table 1, we give the measured isotopic ratios, corrected for contamination, and isotopic discrimination of the mass-spectrometer. Two determinations have been performed at 600 MeV, and one at 21 GeV. The first three ratios give the relative composition of the four isotopes with the largest cross-sections. These are the most precisely measured. As we need the radioactive isotope ⁴⁵Ca (*t*_{1/2} = 165 d) for deriving absolute cross-sections, we also give two ratios relative to this radioisotope. The larger uncertainties in this case are due to both the smaller cross-sections of ⁴³Ca and also to the scandium contribution to the mass-45 peak.

Because ⁴³Ca is pure β⁻-emitter, measurements of the cross-sections are difficult and few have been reported; 1.20 ± 0.13 mb is given at 660 MeV, 1.4 ± 0.2 mb at 730 MeV, and 0.69 ± 0.14 mb at 24 GeV (refs 6, 7, 8 respectively). We do not know of a more recent determination. The good agreement between the values at 660 and 730 MeV gives

Table 1 Ratios of cross-sections for calcium production by spallation of iron, measured by mass-spectrometry

	⁴¹ Ca/ ⁴² Ca	⁴³ Ca/ ⁴² Ca	⁴⁴ Ca/ ⁴² Ca	⁴² Ca/ ⁴⁵ Ca	⁴⁶ Ca/ ⁴⁵ Ca
600 MeV	0.36 ± 0.05	1.18 ± 0.11	1.24 ± 0.14	9.1 ± 2.0	0.27 ± 0.07
21 GeV	0.42 ± 0.06	1.08 ± 0.10	1.10 ± 0.15	9.5 ± 2.8	0.23 ± 0.07

have derived and reported here, although not of the precision we would like, allow a safe enough conclusion on the Ca source abundance.

The experimental procedure has been described elsewhere^{4,5}. We recall that ultra-high purity iron targets were irradiated by protons from the two CERN accelerators (600 MeV and 21 GeV); the spallation products were then chemically separated, and their isotopic composition determined by mass spectrometry. The cross-sections of stable isotopes were obtained with respect to those of radioactive isotopes, measured by classical nuclear physics techniques.

One of the major problems in measuring small quantities of stable isotopes is the contamination by natural impurities.

us some confidence but a new measurement, at least at high energy, would be desirable. Combining these values with our measured isotopic ratios, we derive the absolute cross-sections given in Table 2. These are cumulative cross-sections—including the contributions from short-lived isobars.

The data of Simpson *et al.*² were obtained at ~ 500 MeV m⁻¹. Taking into account solar modulation and interstellar energy loss, the relevant cross-sections should be taken, typically, around 1 GeV. On the basis of known excitation functions, cross-sections at this latter energy are expected to be of the same order as, or slightly larger than those at 600 MeV. Our values are considerably smaller than those adopted by Simpson *et al.*² (70 mb for total Ca,

Table 2 Experimental cross-sections (mb) for calcium production by spallation of iron, compared with Silberberg and Tsao's calculations

	⁴¹ Ca	⁴² Ca	⁴³ Ca	⁴⁴ Ca	⁴⁵ Ca	⁴⁶ Ca
600 MeV	3.9 ± 1.2	10.9 ± 2.7	12.9 ± 3.1	13.5 ± 3.0	1.20 ± 0.13*	0.32 ± 0.09
600 MeV calc.†	2.16	11.82	11.60	12.11	1.23	0.39
21 GeV	2.8 ± 1.2	6.6 ± 2.3	7.1 ± 2.5	7.2 ± 2.5	0.69 ± 0.14*	0.16 ± 0.06
> 2.3 GeV calc.†	3.11	15.68	13.98	13.20	1.24	0.35

*Normalisation, ref. 6 (660 MeV), ref. 7 (24 GeV).

†Ref. 3.

27 mb for ^{42}Ca). At 600 MeV, they agree quite well with those calculated by Silberberg and Tsao's formula³, also given in Table 2. The large discrepancy at high energy is essentially due to our normalising to the ^{42}Ca cross-section measured by Estrup⁸, which is almost a factor of 2 smaller than the calculated one; the disagreement on isotopic ratios is much smaller.

An independent upper limit of the ^{42}Ca cross-sections can be deduced from our scandium measurements⁴. At 600 MeV we made six determinations of the spallation isotopic ratio $^{45}\text{Sc}/^{46}\text{Sc}$, and four at 21 GeV, at times after irradiation varying from 1 d to 280 d (when about 70% of ^{42}Ca had decayed to ^{45}Sc). We observed no systematic increase of this ratio with time, and all values agreed within 2% at 600 MeV, and 5% at 21 GeV, well within experimental uncertainty. This yields conservative upper limits of the ^{42}Ca cross-sections of ~ 1.7 mb at 600 MeV, consistent with the measured value⁸, and ~ 1.8 mb at 21 GeV. The derived upper limits at high energy for ^{42}Ca and total Ca are thus still lower than Simpson *et al.*'s assumption².

We conclude, therefore, that in spite of the rather large uncertainties of our results, it is very unlikely that the observed cosmic ray calcium abundance can be entirely due to interstellar spallation. Moreover, in secondary calcium, ^{42}Ca , ^{43}Ca and ^{44}Ca should have about the same abundance. Thus, the previous assumption that calcium is present in the source with an abundance ~ 10 –15% that of iron is probably correct, but if one accepts the data of Simpson *et al.*² the source calcium must be mainly ^{42}Ca . This would be an enormous difference with Solar System abundances. In addition, measurement of the isotopic composition of other primary cosmic ray species^{9,11} show that they are all consistent with Solar System composition, although there are claims for some enhancement of neutron-rich isotopes of Ne (ref. 9) and Fe (ref. 2). Thus, one would have to find a process of nucleosynthesis which would not only make more ^{42}Ca than ^{40}Ca but at the same time leave all other elements with 'normal' composition. This seems to be quite difficult and considering the low statistics of the calcium measurement of Simpson *et al.*², it seems reasonable to wait for further confirmation of the strange isotopic composition of cosmic ray calcium.

The experimental work was carried out while I was with Laboratoire René Bernas, Orsay, France.

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Received 31 October; accepted 1 December 1977.

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Interpretation of observed cosmic microwave background radiation

ALFVEN and Mendis¹ concluded recently that dust grains in galaxies rendered the Universe opaque to the cosmic microwave background at a red shift of $z = 40$, instead of the generally accepted value of $z = 1,500$. This is highly significant because the microwave background is usually believed to be evidence for a

very hot, dense phase of the Universe². If we can see no further back than $z = 40$, then one objection to nonstandard³ (no big bang) cosmological models is removed. I present here some arguments to show that their assumptions are unreasonable and that a proper calculation in the standard big bang model shows that dust grains are transparent to the microwave background.

Alfven and Mendis¹ calculated the opacity of galactic dust grains by integrating over the metagalaxy at one epoch. A proper treatment would integrate back in time from the present epoch. This alone reduces their estimate of the opacity by a factor of ~ 5 . They also assumed no evolutionary effects in their calculations. But the dust grains are thought⁴ to consist of CH_4 , NH_3 and H_2O with silicate or graphite cores. The mantles sublime⁴ at 100 K, which was the temperature at epoch $z = 36$. The remaining cores have an absorption cross-section roughly 10 times lower⁵ than the ice-coated grains. Another important evolutionary effect concerns the nucleosynthesis of the elements that make up the grains. In the standard big bang model, elements as heavy as oxygen and silicon could not be synthesised in the big bang itself⁶. They must have been synthesised in stars, most likely at an epoch no earlier than $z = 65$. Thus dust grains could not have existed in significant amounts before this era. This will be discussed in more detail later.

Alfven and Mendis¹ calculated the opacity for 1.8 mm microwaves, which they used as the representative wavelength of the cosmic black body radiation. Galactic dust grains, however, are more transparent to longer wavelengths. Observations of the radiation have been made at varying wavelengths⁷ up to 73.5 cm, for which the expected opacity of the grains is less by a factor of 400.

The value of the deceleration parameter q_0 is not well known. Alfven and Mendis used $q_0 = 0.03$. If $q_0 = 0.5$ (flat space), then the opacity of dust grains is reduced by a factor of 4 for a given epoch z . This is because a large deceleration means that the cosmic expansion was faster in the past, so that a ray of light spent less time in any given epoch. A value of 0.5 for q_0 does not contradict any data.

The optical depth τ , for a given wavelength λ_0 along a path from the present era to a past epoch of red shift z_0 , is given by

$$\tau(\lambda_0) = \int_0^{z_0} n(z)\sigma(\lambda)dl \quad (1)$$

where σ is the scattering or absorption cross-section per grain, and n is the number density of the grains. The number of dust grains in a comoving volume remains constant, except for evolutionary changes. As the volume increases as $(z+1)^{-3}$, the number density increases as

$$n(z) = (z+1)^3 f(z) n(0) \quad (2)$$

where $f(z)$ contains all evolutionary effects such as the nucleosynthesis of elements that make up the grains, and is normalised to $f(0) = 1$.

In a matter-dominated Friedmann universe (standard big bang model), a straightforward but messy calculation shows that we can express dl as

$$dl = -cdt = (c/H_0)(z+1)^{-2}(1+2q_0z)^{-1/2} dz \quad (3)$$

Starting with a measurement of $\sigma(\lambda)$ at $\lambda = 0.55 \mu\text{m}$, Alfven and Mendis¹ assumed that $\sigma(\lambda) \propto 1/\lambda$ and extrapolated $\sigma(\lambda)$ to $\lambda_0 = 1.8 \text{ mm}$. Detailed models of the grains⁵ support this functional dependence in a rough way. The evaporation of the grain mantles, however, requires that the opacity drops sharply when the temperature rises above 100 K. This occurred for all epochs

before $z = 36$. As $\lambda \propto R \propto (z+1)^{-1}$ where R is the cosmic scale factor, we can write

$$\sigma(\lambda) = (z+1)g(z)\sigma(\lambda_0) \quad (4)$$

where

$$g(z) = \begin{cases} 1.0 & z < 36 \\ 0.1 & z > 36 \end{cases} \quad (5)$$

Combining equations (1), (2), (3) and (4) we obtain

$$\tau(\lambda_0) = (c/H_0)n(0)\sigma(\lambda_0) \int_0^{z_c} (z+1)^2 f(z) g(z) (1+2q_0 z)^{-1/2} dz \quad (6)$$

At optical wavelengths, the optical depth of our Galaxy parallel to its polar axis is roughly⁸ 0.5. Let D , S_g and n_g be the thickness, area and dust density of the dusty region of our Galaxy. Then

$$Dn_g\sigma(0.55 \mu\text{m}) = 0.5 \quad (7)$$

and

$$n(0) = DN_{og}S_g n_g \quad (8)$$

where N_{og} is the number density of galaxies. Alfven and Mendis used values of $S_g = 5 \times 10^{45} \text{ cm}^2$ and $N_{og} = 1.2 \times 10^{-75} \text{ cm}^{-3}$, both of which are reasonable estimates. Combining these values with equations (7), (8) and $\sigma(\lambda) \propto 1/\lambda$ gives

$$n(0)\sigma(\lambda_0) = 1.65 \times 10^{-33}/\lambda_0(\text{mm}) \text{ (in cm}^{-1}\text{)}. \quad (9)$$

For⁹ $H_0 = 55 \text{ km (s-Mpc)}^{-1}$,

$$c/H_0 = 1.6 \times 10^{28} \text{ cm}, \quad (10)$$

which is the same value Alfven and Mendis used for R_0 .

The only thing needed to finish the calculation of the opacity of the galactic dust grains is the evolution of dust grain density, represented by the function $f(z)$. As dust grains are composed mostly of heavy elements, restrictions on the heavy element abundances will restrict grain densities. Observations of external galaxies by Strom *et al.*¹⁰ show gradients of metal abundances, with the outer regions containing fewer heavy elements than the nuclei. This can be explained¹¹ only by assuming that stellar formation took place during galaxy formation, not before. As stars formed in a predominantly gaseous infall, their nucleosynthesis and subsequent deaths as supernovae (or their stellar winds) enriched the galactic cores more so than the outer regions. I assume that before the Galaxy formed, there was some initial abundance of heavy elements from pregalactic stars. To estimate this abundance, I use the observed heavy element abundance of the poorest metal containing globular clusters, since they are thought to be the oldest objects that can be seen¹². Their metal abundances are roughly 1/300 that of the Sun¹², so $f(z > z_g) = 0.003$, where z_g is the epoch of Galaxy formation. If we assume that our Galaxy started as a spherical cloud that collapsed to a disc, the observed mass and radius of the Galaxy gives a free fall time of roughly $(r^3/GM)^{1/2} \approx 100 \text{ Myr}$. As the epoch $z = 65$ occurred about 100 Myr after the big bang, the galaxies couldn't form earlier than the epoch $z = 65$. Thus an upper bound for $f(z)$ is

$$f(z) = \begin{cases} 1.0 & z < 65 \\ 0.003 & z > 65 \end{cases} \quad (11)$$

Combining equations (5), (6), (9), (10) and (11) enables us to do the integral and calculate $\tau(\lambda_0)$. Recent measurements¹³ of the black body anisotropy used a wavelength of $\lambda_0 = 9 \text{ mm}$. At

$z_c = 1,500$, the generally accepted value of decoupling, the optical depth due to absorption by galactic grains is $\tau(9 \text{ mm}) = 0.18$ for $q_0 = 0.03$ and $\tau(9 \text{ mm}) = 0.05$ for $q_0 = 0.5$. Thus most of the light emitted at the time of decoupling has reached us unscattered by intervening dust.

To conclude, I have calculated the opacity of galactic dust grains to the microwave background radiation from the time of decoupling at $z_c = 1,500$ to the present in the standard big bang model. I have estimated evolutionary effects on grain opacity and abundance, conservatively overestimating the expected grain density. At typical wavelengths used in studying the microwave background, I have shown that the optical depth of the grains is only 0.18 for $q_0 = 0.03$ and is 0.05 for $q_0 = 0.5$. Thus the microwave background can give us information on an early dense phase of the Universe.

I thank Drs C. McKee, A. Buffington, R. Muller and G. Smoot for reviewing this paper; Drs C. Orth and T. Tyson for helpful discussions, and Dr H. Alfven for a very stimulating discussion. This work was supported by NASA.

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Received 28 July; accepted 16 November 1977.

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Plasma fluxes between ionosphere and protonosphere

PLASMA flux between ionosphere and protonosphere is important for our understanding of ionospheric-magnetospheric coupling. Attempts have been made to deduce the H^+ flux between ionosphere and protonosphere from incoherent scatter observations of O^+ densities and vertical velocities in the topside ionosphere^{1–4}, but theoretical studies⁵ have suggested that the O^+ flux is not necessarily related in a simple way to the H^+ flux and may even at times be in the opposite direction. More direct estimates of average daytime and night-time fluxes have been calculated from a limited data base of whistler observations of tube content⁶, while topside electron density profiles have also been used⁷.

The ATS-6 radio beacon experiment⁸ provides a method of estimating integrated net ionospheric-protonospheric fluxes on a continuous basis from the temporal changes in protonospheric content. The receiving station at Aberystwyth (52.42°N, 4.05°W) for transmissions from the ATS-6 satellite (geostationary above 35°E) allowed determination of both the plasmaspheric group delay and the ionospheric polarisation rotation. The former is related directly to the total electron content (N_T) along the slant path from satellite to receiver while the latter gives a measure of the Faraday electron content (N_F) along the ionospheric part of the path.

Model studies^{15–17} have shown that using an average longitudinal gyrofrequency for the path, evaluated at a fixed height in the range 350–420 km, in the conversion of polarisation rotation to Faraday content produces values which give a measure of the electron content up to an effectively constant altitude between 2,000 and 3,000 km. For geometry applicable to the present study the height sensitivity of the longitudinal

gyrofrequency is small, differing, over the height range 300 to 500 km, by less than $\pm 1.3\%$ from the chosen value for 420 km.

From the difference between N_T and N_F measurements made to a sufficient degree of accuracy, the electron content of the protonospheric part of the path (N_p) can be obtained. In practice, N_p is a measure of the content from $\sim 2,500$ km altitude to the plasmapause, estimated to an absolute accuracy within $\pm 15\%$, but with much better relative accuracy allowing small temporal changes to be studied. The protonospheric content expressed as a percentage of the total ranges in general from some 15–20% by day to 30–40% by night.

Temporal changes of protonospheric content are indicative of the filling and draining of this region where production and loss processes are unimportant so that the ionospheric–protonospheric flux can be obtained from the temporal gradient of the protonospheric content (dN_p/dt). It should be noted that the protonospheric content measured by the ATS-6 experiment refers to a slant path to the satellite which intersects a wide range of L -value flux tubes, as shown in Fig. 1 for Aberystwyth geometry up to $L = 4$. The protonospheric flux estimated from ATS-6 measurements is thus an integrated parameter over these tubes in contrast to the more direct measurements of flux, essentially along a particular field tube, given by the whistler and incoherent scatter techniques.

Monthly median diurnal values of N_p have been used to estimate the temporal rate of change of this parameter (dN_p/dt), calculated for each hour. To reduce the scatter in the data points, the N_p values were first filtered using a 3-h running mean before calculating the gradients. The diurnal variations of the fluxes for December 1975 and January 1976, representing northern hemisphere winter, and June and July 1976 corresponding to summer conditions are shown in Fig. 2. Positive values are indicative of an upwards flux filling the tubes with negative values corresponding to downwards depletion. The highly intermittent nature of the beacon transmissions, particularly while the satellite was eclipsing, have prevented reliable flux data being obtained for the equinoctial season.

To help explain some of the observed features of the diurnal variations of the integrated flux, the sunrise and sunset times, in both local (47°N , 22°E) and conjugate (35°S , 37°E) hemispheres at 350 km, at the ionospheric base of the $L = 2$ flux tube, which intersects the ATS-6 ray path at $\sim 5,000$ km altitude, are marked on Fig. 2.

In interpreting Fig. 2 it must be remembered that the satellite crosses many field lines at different heights, the ionospheric feet of which become sunlit at different times. Figure 1 indicates the range of latitudes of the ionospheric terminations of the field lines within the plasmasphere below $L = 4$, but taking account of the longitudinal range as well, the spread of sunrise and sunset times for the feet of the relevant field lines in both local and conjugate hemispheres can exceed ± 1 h about the times shown in Fig. 2. A second factor to consider is the time constant between an increase in ionospheric ionisation and the appearance of enhanced protonospheric plasma. A time constant ~ 1 h has been suggested for the diffusive barrier at the O^+/H^+ transition height⁹, while the travel time of ions

Fig. 1 Geomagnetic field line configuration for ATS-6 to Aberystwyth ray path.

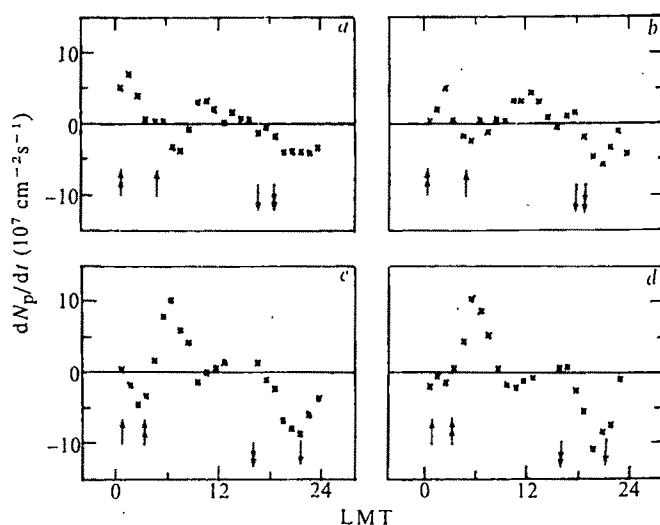
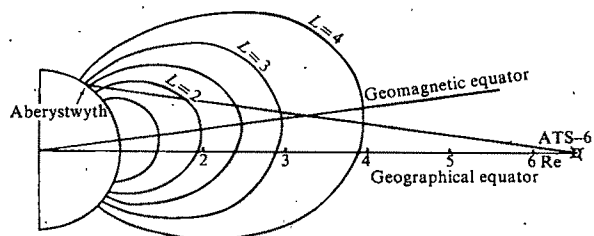


Fig. 2 Diurnal variations of the integrated protonospheric flux (dN_p/dt) for Aberystwyth for *a*, December 1975, *b*, January, *c*, June and *d*, July 1976. The arrows mark the times of sunrise and sunset in both local and conjugate hemispheres at the terminations at ionospheric height (350 km) of the $L = 2$ field line which intersects the ray path at $\approx 5,000$ km altitude. \uparrow , local sunrise; \downarrow , local sunset; \uparrow , conjugate sunrise; \downarrow , conjugate sunset.

to the protonosphere even at thermal speed may considerably exceed 1 h depending on the field line path. Thus the overall time constant may be of the order of several hours.

Considering first the Northern Hemisphere summer curves represented by June and July in Fig. 2, it can be seen that upwards fluxes start some 2 h after local sunrise with maximum values some 2 h after conjugate sunrise. The subsequent decrease in the flux rate may indicate that the relatively short small volume tubes of low L -value (say $L \leq 2$) become well filled so that the upwards flux to them is decreased and the subsequent small increase in protonospheric content occurs on the large volume higher L -value flux tubes¹⁰. The rates of change do not differ significantly from zero in the middle of the summer day when the neutral wind is depressing the F-layer ionisation, but after conjugate sunset a large downwards flux quickly develops. It is interesting that this downwards flux maximises at about the time of the second daytime ionisation peak in the still sunlit local ionosphere. This suggests that the protonospheric depletion at this time is to the conjugate ionosphere and the shape of the flux variation, quickly maximising downwards with subsequent smaller magnitudes, seems to indicate that the short inner tubes are quickly drained. The northern hemisphere winter curves represented by December and January show an upwards flux following sunrise in the local hemisphere and a sunset associated depletion. It is also interesting that a short lived upwards flux can be found around conjugate sunrise for these months when the time separation between sunrise in the two hemispheres is a maximum. This net upwards flux is of short duration, however, and the enhanced losses and effective downward movement of the local ionosphere evidenced by the well-known pre-dawn electron density depletion more than compensate for conjugate induced enhancements. A net decrease in protonospheric content is thus found until after local sunrise.

The average integrated fluxes obtained in the present study show that to within the accuracy of the estimates the upwards flux by day in magnitude and duration is broadly compensated by the downwards flux at night. Park⁶ from whistler observations has estimated average flux magnitudes generally greater than the levels indicated by the present study, with the average daytime upwards flux exceeding the downwards night-time flux by a factor of 2. While the present results are based on monthly median protonospheric content data, the values in

Park's case study refer to a post-storm period during which the protonosphere is being replenished with daytime refilling exceeding night-time draining. Additionally, his observations are for tubes with $L > 4$ while the Aberystwyth ray path crosses tubes with $L \geq 1.7$ and it has been argued that the flux is enhanced for higher L -shells because of the relatively less dense plasma in these larger volume tubes¹⁰.

It can be concluded from this study that both local and conjugate ionospheres play important parts in the filling and depletion of the protonospheric flux tubes; a result supported by recent theoretical work¹¹. Thus in the interpretation of protonospheric content data corresponding to observations along the line of sight to a geostationary satellite, ionospheric-protonospheric interactions in both hemispheres must be considered. This conclusion enables us to account for the significant differences found between ATS-6 observations of protonospheric content made in Europe and USA. A set of protonospheric content data for July 1974 to May 1975 from ATS-6 observations made at Boulder, USA (40°N, 105°W) has been published¹². These monthly median values of N_p converted to local mean time show a daytime minimum in protonospheric content in winter and very low values in summer. By contrast, the Aberystwyth data (albeit for a later year but still near solar minimum) have a daytime maximum at all seasons with winter night-time magnitudes comparable to those at Boulder, but in summer the European content can be more than double that of the America station. A detailed examination¹³ shows that, in addition to the effects of observational sensitivities, these differences may be explained in terms of ionospheric-protonospheric interactions in both hemispheres, remembering that the nonalignment of the geographic and geomagnetic axes results in an ionosphere conjugate to Boulder which is sub-Antarctic in location (57°S, 105°W). The anomalous behaviour of the continuously sunlit Antarctic ionosphere in local summer (December-January) under the influence of the neutral wind is well documented¹⁴, and this factor, together with the long winter nights and the generally higher L -shells intersecting the Boulder ray path, may account for the differences between the European and American protonospheric contents.

We thank Professor Sir Granville Beynon, FRS for his interest in the planning of ATS-6 observations in UK. The cooperation of Dr E. N. Bramley and Mr L. J. Martin of Appleton Laboratory and Dr J. K. Hargreaves and Dr S. Ganguly of University of Lancaster in the ATS-6 project is gratefully acknowledged. This work has been sponsored by SRC through Grant SG/R/052 with additional support from AFGL, Massachusetts through EOARD, London: Grant AFOSR-72-2267.

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Post-geomagnetic storm protonospheric replenishment

THE response of the ionosphere to geomagnetic storms is well documented¹⁻³, although the complex interaction of the various physical mechanisms is not yet fully explained. However, the response of the overlying protonosphere to geomagnetic activity, a topic important to the understanding of ionospheric-magnetospheric coupling has not been widely studied. A direct study of tube content recovery obtained from whistler observations during an 8-d period in June 1965 following a sudden commencement storm has been published by Park⁴, although theoretical work⁵ has given a value for the replenishment time constant which is larger than that observed. We report here the results of protonospheric content observations during a period where a major sudden commencement storm was followed by an exceptionally long period of quiet allowing complete dynamic saturation of the protonospheric flux tubes to be reached.

The ATS-6 radio beacon experiment⁶ carried out at Aberystwyth (52.42°N, 4.05°W), while the geostationary satellite was stationed at 35°E longitude, allowed estimates to be made of both the plasmapheric total electron content (N_T) and the ionospheric Faraday electron content (N_F) to sufficient accuracies that the protonospheric electron content along the slant path (N_p) could be obtained from the difference. We report here the results of protonospheric electron content measurements during the period 28 April to 21 May 1976. Hourly values of N_p available throughout the period are plotted in Fig. 1. Also shown for comparison, recurring for each day, is a plot of the diurnal variation of the averaged hourly N_p for May 1976. Values of the 3-h K_p index are presented above the corresponding N_p data. The period starts with 4 d in which the geomagnetic activity reaches $K_p=4_+$ late on 29 April and N_p shows a fluctuating pattern, particularly on 30 April and 1 May when the diurnal variation is almost masked by apparently random variations. A major storm with sudden commencement at 1828 UT on 2 May and K_p reaching 8_+ is preceded by a sharp increase in N_p around midday to be followed by a depletion with N_p reaching very low values on the morning of 3 May and even lower magnitudes around midnight of that day. In fact this value $1.3 \times 10^{16} \text{ m}^{-2}$ was the lowest N_p value found at Aberystwyth during the 9-month observing period. Three days follow with substantial breaks in the N_p data due to non-continuity of the satellite transmissions, but the values plotted show the trend of a replenishing protonospheric content which is continued until about 16 May during a time of quiet geomagnetic activity. Superposed on this trend is a regular diurnal variation with increasing amplitude as the quiet period continues. From 16 to 19 May the geomagnetic activity is very low and N_p is consistently above average with a continuing clear large diurnal variation. It therefore seems that the depleted protonosphere of 3 May requires some 14 d of quiet conditions with steady diurnal partial filling and draining to reach a quasi-equilibrium situation about 16 May. Thus from 16 to 18 May the protonospheric content for ATS-6 to Aberystwyth geometry presents its saturated dynamic equilibrium pattern for quiet geomagnetic activity with values maximising around $7 \times 10^{16} \text{ m}^{-2}$ during daytime filling and depleting to about $4 \times 10^{16} \text{ m}^{-2}$ at night. The maximum and minimum values of N_p for each day both increase steadily from the storm depletion with the daytime filling more than compensating the night-time draining for some 12-14 d. In the saturated dynamic equilibrium situation of 16-18 May the maximum daytime protonospheric electron content (N_p) makes up some 20% of the total electron content (N_T) as opposed to only 11% on the day following the sudden commencement.

The first point to note from this study of N_p changes is that the regular diurnal variation, with early morning minimum

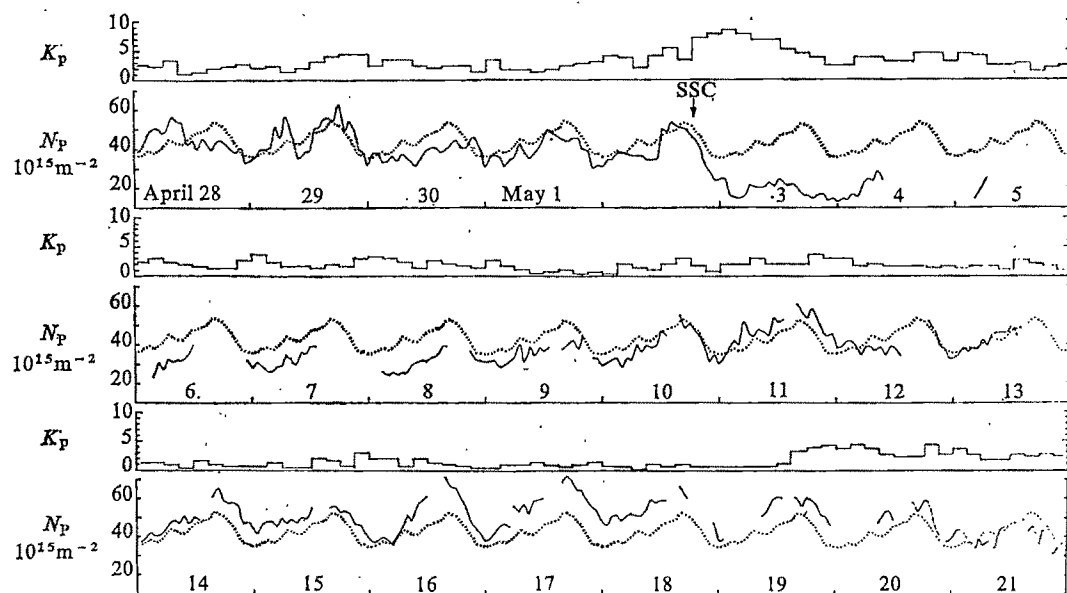


Fig. 1 Protonospheric content variations from 28 April 1976 to 21 May 1976. The dotted curve displays the mean diurnal variation for May 1976 recurring each day. Also plotted are the three-hourly values of the planetary geomagnetic index K_p .

and afternoon maximum, is essentially a quiet time phenomenon. When K_p values exceeding 3 are found over a period of several days the diurnal pattern of N_p becomes irregular with noiselike quasi-random fluctuations which tend to mask the diurnal component of the variation.

Enhanced geomagnetic activity accompanying a sudden commencement is associated with an increase in the daytime protonospheric content. This result is consistent with that reported by Poletti-Liuzzi *et al.*⁷. It is well known that ionospheric electron content can show a significant positive effect during daytime following sudden commencement. This increase is associated with the raising of the ionisation under the influence of the magnetospheric electric field penetrating to the mid-latitude ionosphere and also neutral winds, possibly reversed in direction and blowing equatorwards in response to enhanced temperatures in the auroral zone. It is thus to be expected that the increase in ionospheric plasma will be reflected in enhanced protonospheric content. An additional factor which may cause some enhancement in slant N_p is the expansion of the plasmasphere in the afternoon sector in response to increased magnetospheric convection.

The rapid depletion in N_p as the line of sight moves into the night side is consistent with the corresponding drop in ionospheric electron content and peak density reported by many workers. The main cause of this latter decrease is almost certainly linked to changes in thermospheric circulation reflected in an increased N_2/O ratio⁸, but the enhanced magnetospheric electric field probably plays a part in depressing the night-time ionisation and thus reducing the ionospheric electron content and hence protonospheric content. The additional factor which contributes to the lowering of the night-time N_p during the main phase of the storm is the contraction of the plasmapause radius caused by the convection electric field. For example, Park⁴ found that the plasmapause moved in to $L \approx 2.4$ several hours after local midnight during a storm in which K_p reached 7.

The slow refilling of the protonosphere after the storm depletion took ~ 14 d to reach saturation during the quiet conditions in May. The results of the whistler studies reported by Park⁴ indicate time constants to reach equilibrium ranging from ~ 1 d at $L=2.5$ to 8 d at $L=4$. The storm of 2 May 1976 with K_p maximising at 8+ was more intense than that discussed by Park so that the protonospheric depletion may have been more complete in our observations where the lowest N_p value ever found occurred, however, in terms of season and solar minimum epoch the data are comparable. Park's observations were interrupted by substorm activity after 8 d so that he was unable to study possible saturation beyond $L=4$. However, in

the present observations the period of quiet extends to ~ 17 d allowing complete equilibrium along the slant path to be reached after ~ 14 d when the plasmapause radius had extended to its furthest possible quiet time position. Murphy *et al.*⁵ calculated a post-storm saturation time for the H^+ tube content which was much greater than the experimental estimate of Park for $L=3$ at solar minimum—they reported a refilling time greater than 8 d at equinox.

After ~ 7 –8 d the N_p magnitudes and their diurnal variation become comparable with the monthly average values suggesting that the median protonospheric content measured by the ATS-6 experiment represents the integration over flux tubes up to $L \sim 4$.

We thank Professor Sir Granville Beynon, FRS, for his interest in planning ATS-6 observations, and Dr E. N. Bramley and Mr L. J. Martin (Appleton Laboratory) and Drs J. K. Hargreaves and S. Ganguly (University of Lancaster) for their cooperation in the ATS-6 project. This work was sponsored by SRC (grant SG/R/052) with additional support from US AFGL, through EOARD, London (grant AFOSR-72-2267).

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Received 19 August; accepted 5 December 1977.

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Possible climatic and biological impact of nearby supernovae

OZONE plays a very important part in atmospheric radiative transfer. The absorption of the solar ultraviolet radiation by ozone is the dominant heating mechanism in the stratosphere. At thermal infrared wavelengths the main ozone contribution comes from the $9.6 \mu\text{m}$ band. Clark *et al.*¹ were unclear, however, whether the supernovae removal of ozone

from the atmosphere of the Earth would heat or cool the surface of the planet. There is evidence, reported here, which enables a more precise statement to be made of the effect upon the global Earth. The reduction in the concentration of ozone will cool the stratosphere, troposphere and surface layers of the Earth.

An estimate of the ozone effect upon the global temperature structure may be made through radiative equilibrium models in which the convective adjustment to the lapse rate is used to approximate the dynamical effects (see, for example, Manabe and Wetherald²). The atmospheric model is one dimensional and is allowed to reach an equilibrium structure with a time marching procedure. For the assumed atmospheric composition and initial temperature structure, the solar and thermal heating/cooling rates are calculated at each level and the net heating/cooling used to determine the temperature at each altitude at time $t + \Delta t$. If the computed temperature lapse rate exceeds the maximum assigned value of -6.5 K km^{-1} , then a convective adjustment occurs with a vertical energy flux sufficient to yield that pre-assigned maximum lapse rate. The time marching procedure is continued until equilibrium is reached at each level in the atmosphere, which is typically 300 d with a time step of 1 d. The final atmospheric structure is independent of the initial temperature profile.

Table 1 gives examples of predicted changes due to the reduction in ozone amount. The small difference in the exact magnitude of the change results from minor differences in the radiative schemes and cloud formulations used by the individual authors. In physical terms a reduction in ozone leads to a decrease in stratospheric solar absorption. The cooling of this region will reduce the downward longwave flux in the $9.6 \mu\text{m}$ band and therefore cool both the troposphere and surface. But the changes in the tropospheric temperatures will be much smaller than the stratospheric effects where ozone plays a dominant part (Table 2).

Table 1 Surface temperature changes due to reduced ozone

Ozone reduction (%)	Temperature change (K)	Ref.
25	-0.34	3
50	-0.3	5
	-0.4	4
100 (No ozone)	-0.5	5

The possible biological consequences of the reduction in atmospheric ozone through these supernovae events will be catastrophic for certain species, as DNA, which carries the genetic information of the cell, is easily modified by exposure to ultraviolet radiation. While living cells have a significant enzymatic capacity for repairing ultraviolet damage to their DNA, living things are in a delicate balance between the continual photochemical destruction of cellular components by solar radiation and their biological repair. If this balance is upset by exposure to increased amounts of ultraviolet radiation which would result from the supernovae events postulated by Clark *et al.*¹, the organism will be injured and may die.

The climatic implications of these supernovae events are also likely to be equally catastrophic. It is generally accepted that a reduction in global temperature of 1K is necessary to produce an ice age (see, for example, ref. 7), which would, of course, hide the larger changes that occur on a local scale. The calculations produced here may not suggest an immediate ice age would follow the complete destruction of atmospheric ozone; but these calculations have been performed with a simple model which does not include the feedbacks that may result from the dynamical

Table 2 Atmospheric temperature changes due to reduced ozone

Pressure (mbar)	Temperature change (K)		
	30% decrease ⁴	50% decrease ⁵	No ozone ⁵
22	-7	-11	-69
230	-0.4	-0.5	-0.6
1,000	-0.2	-0.3	-0.5

coupling between the stratosphere and troposphere. Some insight into these feedback mechanisms has been given by Bates⁸ who suggests the large changes in stratospheric temperature, such as those indicated in Table 2 due to the reduction in ozone amount, would greatly affect the stratospheric meteorology and in particular the static stability of this region. Furthermore, Bates⁸ shows that the ultra-long wave geopotential field in the troposphere is very sensitive to these stratospheric changes indicating a coupling between these atmospheric layers, and a further possible mechanism by which these stratospheric ozone changes would affect the terrestrial climate. There would seem to be little doubt the encounters with nearby supernovae, causing a major reduction in the atmospheric ozone content, would initiate an ice age as Clark *et al.*¹ postulated. This is a further example of an astronomical influence on the terrestrial environment.

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Received 6 October; accepted 2 December 1977.

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Surface topography as a nonstationary random process

TOPOGRAPHY is often considered as a narrow bandwidth of features covering the form or shape of the surface. After detailed study of many measurements we consider that as well as the possibility of a dominant range of features there is always an underlying random structure where undulations in surface height continue over as broad a bandwidth as the surface size will allow. We consider this a result of many physical effects each confined to a specific waveband but no band being dominant. We invoke the central limit theorem and show through Gaussian statistics that the variance of the height distribution of such a structure is linearly related to the length of sample involved. In another form, the power spectral density, this relationship is shown to agree well with measurements of structures taken over many scales of size, and from throughout the physical universe.

Van Deusen¹ noted that the power spectral densities of a variety of natural and artificial surfaces fall off as the square of the angular frequency. It has since been observed particularly in the longer wavelength irregularities²⁻⁴ that many engineering surfaces exhibit similar spectra. Similarities in the structure of surfaces of such disparate scales of size suggest the existence of a common natural law underlying the phenomena. The specific form of the spectra further suggests that a sample of finite length taken from such a surface will never, however long, completely represent its properties. Real surfaces cannot

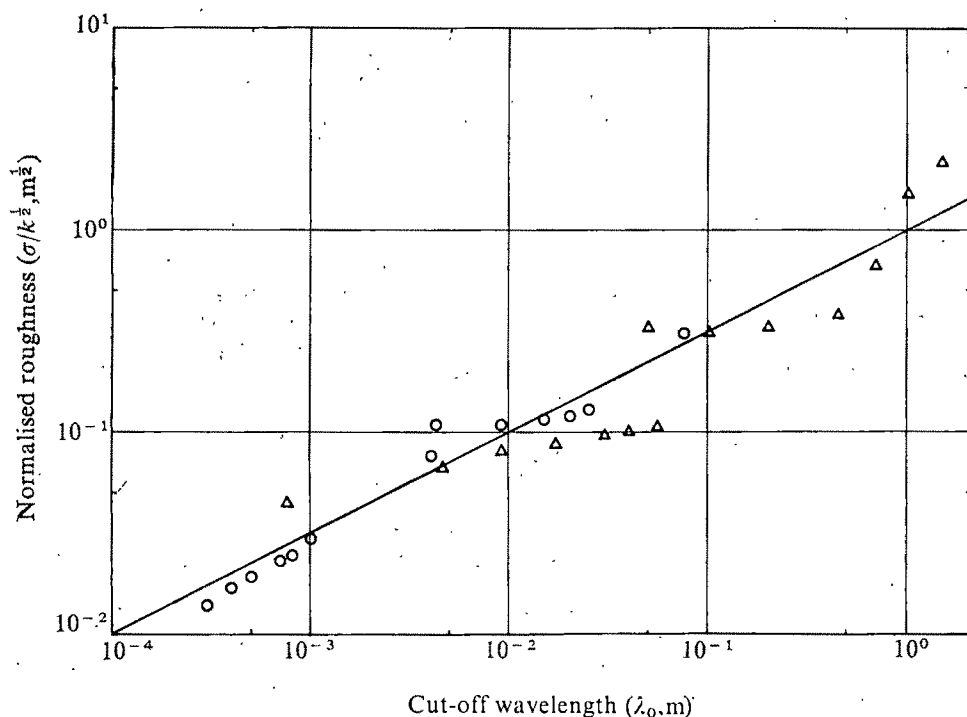


Fig. 1 The experimental points show the increase in normalised r.m.s. roughness ($\sigma/k^1, m^1$) with cut-off wavelength (λ_0, m) observed from two different topographies. The solid line represents the theory given by equation (1). ○, Grit blasted surface, $k(m)=1.7 \times 10^{-8}$ (ref. 7). △, Planed surface, $k(m)=4.4 \times 10^{-12}$ (ref. 2).

therefore be treated as stationary random processes, as has previously been supposed^{5,6}.

A further property of such a spectrum is that its energy increases rapidly with increasing wavelength. The area under the spectrum, which is the variance of the height distribution or, in engineer's terminology, the square of the r.m.s. roughness, is thus most heavily influenced by the longest wavelengths in the sample. This in turn suggests that the roughness must be related in some way to the length of sample or to the measured bandwidth, a hypothesis supported by experimental evidence^{2,7,8} (Fig. 1).

To form such a surface structure we postulate a process resulting from many random causes without preference for any one wavelength or waveband. From the central limit-theorem we would expect the amplitude distribution of a sample from such a process to be Gaussian. This is true even if the result of each individual effect forming the process is non-Gaussian. Furthermore, because the process cannot be confined to any one bandwidth, the variance of the Gaussian height distribution will be a function of the length of sample considered, or, equivalently, the bandwidth of features represented.

With a surface of a given finite area, if the heights are measured sufficiently closely together then an arbitrarily large sample can be obtained. Its extreme values, however, would not be very large and will therefore be a function not of the sample size, as for a discrete normal variate, but of the area over which the sample was taken. This is because in the physical world we do not expect things to vary by vast amounts in short periods or distances: a consequence, broadly speaking, of the energy necessary to create large changes in values. Large changes in surface height on Gaussian surfaces are possible but they tend to occur over large distances. This argument suggests that in physical situations a Gaussian stationary process is in fact a contradiction in terms.

Suppose the standard deviation σ of the height distribution is a function of the length of sample L , that is, $\sigma = f(L)$. To investigate the effects of increasing sample length on r.m.s. roughness we will consider consecutive lengths L_1 and L_2 . Wiener⁹, considering the analogous problem of the wandering of a particle in non-overlapping intervals of time, showed that if the two distributions governing the extent of wandering were independent and Gaussian, then the combined distribution is also Gaussian. Thus giving the standard result of the addition theorem that the variances add to form the variance

of the new distribution. The interesting aspect of this result is that in our case, where the standard deviation is a function of sample length then because

$$\sigma^2 = \sigma_1^2 + \sigma_2^2$$

we can write,

$$f^2(L_1 + L_2) = f^2(L_1) + f^2(L_2)$$

and the only function $f(L)$ which holds for the above equation is a linear one,

$$\sigma^2 \propto L$$

The relationship depends on the independence argued between samples, thus with machined surfaces it is restricted to a certain range of features. In general this range is most evident over the spatially longer features of the topography, the smaller features being dominated by the machining marks; however, many natural surfaces have no such restrictions. In such cases the process involved corresponds to the non-stationary Wiener-Levy process¹⁰, of which the best-known example is Brownian motion. Such a process is continuous but nowhere differentiable, confirming the view⁵ that slopes, curvatures and densities of maxima and minima are not, as was formerly widely believed, intrinsic properties of a surface.

This relationship can be expressed in a spectral form, as the effective high-pass cutoff wavelength λ_0 of a sample must be proportional to its length, that is,

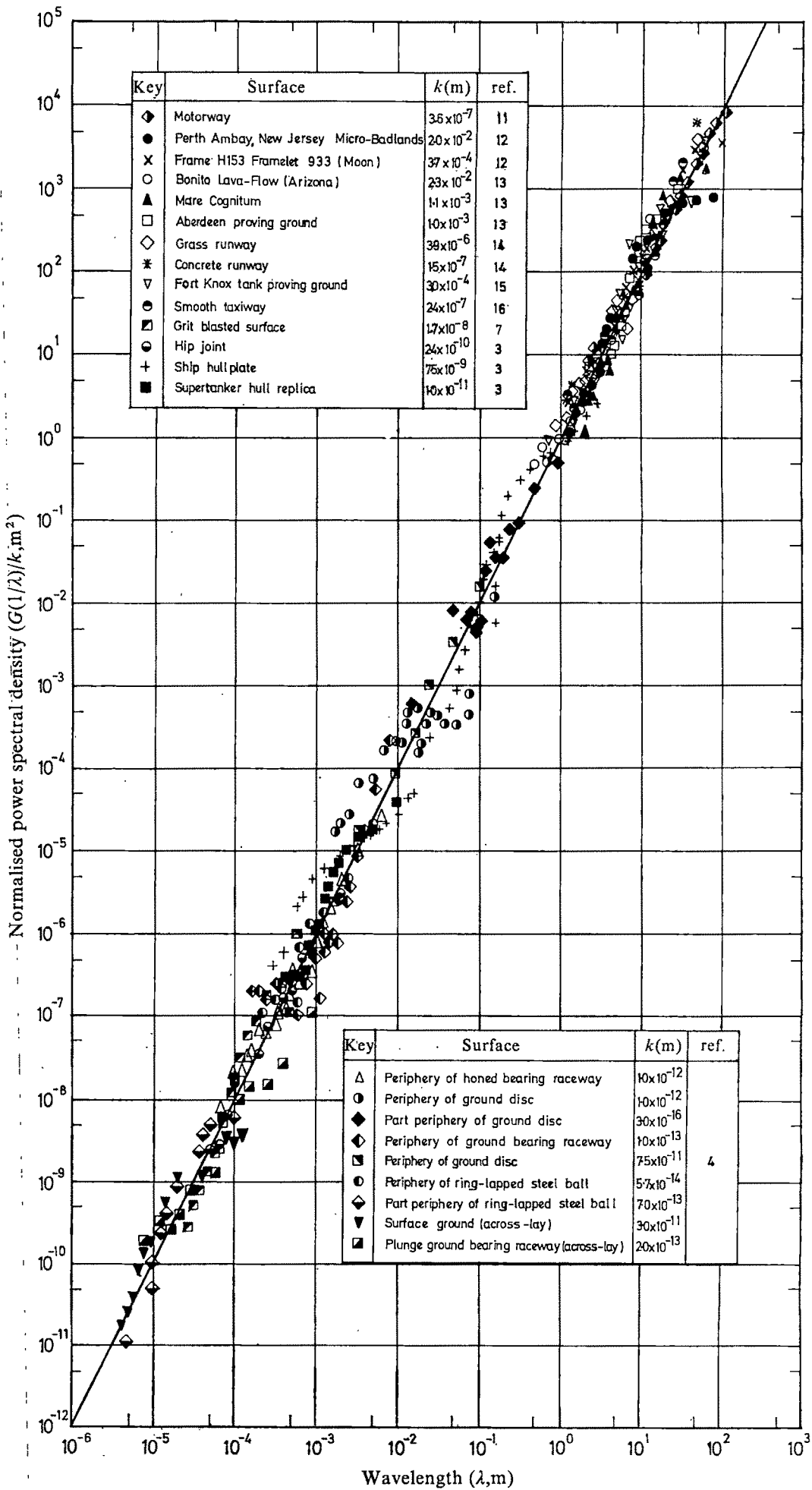
$$\sigma^2 = k\lambda_0 = 2\pi k/\omega_0 \quad (1)$$

where ω_0 is the corresponding angular frequency and k the constant of proportionality, a result confirmed by experiment (Fig. 1). But the area under the power spectral density curve $G(\omega)$ is also the variance of the height distribution:

$$\sigma^2 = \int_{\omega_0}^{\infty} G(\omega) d\omega$$

Combining the above two equations, and assuming

Fig. 2 Variation of normalised power spectral density ($G(1/\lambda)/k, m^2$) with wavelength (λ, m). The graph shows that many different surface topographies existing in the physical universe have a similar form of power spectrum. Note that the spectra available cover almost eight decades of surface wavelength and throughout this range the r.m.s. power increases, to a good approximation, as the square of the wavelength (solid line, equation (2)).



$$\lim_{\omega \rightarrow \infty} G(\omega) = 0$$

which is expected with physical data, then

$$G(\omega) = 2\pi k/\omega^2 = (k/2\pi)\lambda^2 \\ \text{or } G(1/\lambda) = k\lambda^2 \quad (2)$$

in agreement with experiment (Figure 2).

We call k the 'topothesy' of the surface (τοποθεσία, a description of a place or region¹⁷). Its value uniquely defines the statistical geometry of the random components of an isotropic surface for any given range of wavelengths. The topothesy has units of length. It seems able to characterise successfully and completely, examples of surface structures of a wide range of sizes (Fig. 2) drawn from throughout the physical universe.

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Isotope separation by 'Taylor diffusion'

THE axial dispersion of injected diffusible matter in a fluid flowing in steady laminar regime through a tube has been described by Taylor. On this principle, a simple technical device enabling isotopic separation can be constructed. The separation factor for light stable isotopes is higher than that reached by standard methods; solar energy can be used as an energy source for its functioning.

Taylor^{1,2} and later Aris³ have shown that when a soluble substance is introduced into a fluid flowing through a tube in laminar regime, it spreads out under the combined action of molecular diffusion and the variation of velocity over cross-section. The axial dispersion can be characterised by a virtual coefficient of diffusion (K) which is given by

$$K = (a^2 \bar{u}^2 / 48D) + D \quad (1)$$

if the following condition holds

$$(4L/a) \gg (\bar{u}a/D) \quad (2)$$

D is the coefficient of molecular diffusion of the soluble substance assumed to be independent of its concentration; a is the radius of

the tube, L the distance between the point of introduction and measurement and \bar{u} the mean axial velocity of convection.

If the origin of the coordinates ($z = 0$) is chosen at the point where the substance is injected instantaneously, its axial distribution can be described by the Gaussian curve

$$C(z, t) = \frac{1}{2}(\pi K t)^{-1/2} \exp[-(z - \bar{u}t)^2 / 4Kt] \quad (3)$$

which is the solution of the diffusion equation

$$\frac{\partial C}{\partial t} = K \frac{\partial^2 C}{\partial z^2} - \bar{u} \frac{\partial C}{\partial z} \quad (4)$$

with $C(z, t = 0) = \delta(z)$ as initial condition.

Taylor diffusion has been considered as one of the mechanisms of gas mixing in the human lung. Our previous work on gas transport in the bronchial ducts⁴ has led to a straightforward application of Taylor diffusion to separate isotopes⁵.

Each element of separation (Fig. 1) is essentially formed by a simple tube of length L and circular cross-section of radius a , through which a carrier fluid flows in steady laminar regime. At one end of the tube ($z = 0$), pulses of isotopic mixture are injected periodically with a frequency f . The concentration of a single pulse is given by equations (1) and (3), where D is the molecular diffusion coefficient of each isotopic component in the carrier fluid. Concentration curves must crossover and these intersection points define alternate zones in the tube which are enriched in one or other of the isotopes (bottom of Fig. 1). Heavy isotopes spread more than light ones.

If a valve system is used at point $z = L$, enabling the alternate communication between the principal duct and each of the secondary ducts, and if the valve switch times correspond to crossover concentration points, then each secondary tube will be enriched in one particular isotope.

The separation factor α is given by the classical formula⁶

$$\alpha = \frac{n_{1S}n_{2I}}{n_{1I}n_{2S}} \quad (5)$$

In our case n_{1S} and n_{2I} are the number of molecules of isotope 1 and 2 in the upper (S) and lower (I) secondary ducts respectively (Fig. 1). (We assume $n_{1S} > n_{1I}$.)

In order to produce a fast axial dispersion, we chose parametrical values such that

$$\frac{a^2 \bar{u}^2}{48D} \gg D \quad (6)$$

which, together with condition (2) is consistent with Reynolds numbers ensuring laminar flow. If we call K_1 and K_2 the dispersion coefficients given by equation (1) for isotope 1 and 2 we have

$$\frac{K_2}{K_1} = \frac{D_1}{D_2} \quad (7)$$

Where D_1 and D_2 are the molecular diffusion coefficients of isotopes 1 and 2 in the carrier fluid.

Let m_1 , m_2 and m_0 be the molecular weights of isotope 1, 2 and

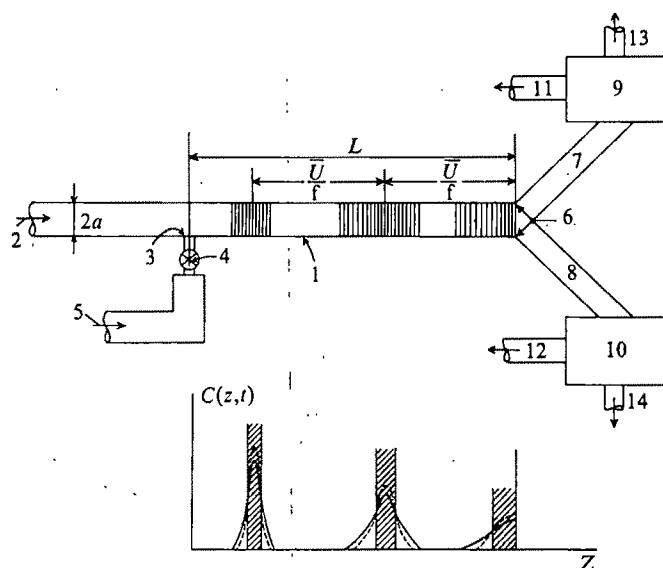


Fig. 1 Isotope separation element: 1, main duct; 2, inflow of carrier fluid; 3, isotopic mixture injection point; 4, injection valve; 5, inflow of isotopic mixture; 6, outflow valve; 7, 8, outflow or secondary ducts; 9, 10, separators of isotopic mixture and carrier fluid; 11, 12, outflow of carrier gas; 13, 14, outflow of isotopic mixture. The concentration of each isotope after $t = L/\bar{u}$ after first injection and expressed as a fraction of the injected concentration. Shaded zones correspond to zones enriched in light isotope.

the carrier fluid respectively. From the Chapman-Enskog classical equation, one can show that

$$K_2/K_1 = (m_2/m_1)^{1/2} [(m_0 + m_1)/(m_0 + m_2)]^{1/2} \quad (8)$$

if $m_2 > m_1$. If $m_1 \approx m_2$, we can write

$$K_2/K_1 = 1 + \varepsilon; \quad \varepsilon \ll 1 \quad (9)$$

When inequality 2 holds, equation (3) can be approximated by a Gaussian distribution and the separation factor is then given by

$$\alpha = 1 + \varepsilon \{ (2\pi e)^{1/2} \operatorname{erf}(1/\sqrt{2}) [1 - \operatorname{erf}(1/\sqrt{2})] \}^{-1} \quad (10)$$

or

$$\alpha = 1 + 1.12\varepsilon \quad (11)$$

Among the different methods of isotopic separation, mass diffusion developed by Hertz^{7,8} also makes use of an auxiliary gas. Later, Maier⁹ used elements consisting of concentric columns separated by a barrier and a feed gas was injected into the innermost column. The faster molecular diffusion of the lighter isotope in the feed gas enables separation.

Both in mass diffusion and in our method, almost all the energy used in the process is related to the separation of the isotopic mixture and the auxiliary fluid. Indeed, in our method, the energy consumption associated with the pipe flow viscous losses is very small. The minimum work required to separate m_1 moles of isotopic mixture and m_2 moles of auxiliary fluid is given by

$$W = RT \left(m_1 \ln \frac{m_1}{m_1 + m_2} + m_2 \ln \frac{m_2}{m_1 + m_2} \right) \quad (12)$$

Where T is the absolute temperature and R the gas constant⁶. However, the proposed method presents three important advantages over mass diffusion: firstly, the simplicity of the element of separation, which is basically constituted by a simple tube; secondly, fluids in their liquid phase can be used, which leads to much smaller plants, and finally, the possibility of having a higher

separation factor. Indeed, in mass diffusion plants, it is recommended that an auxiliary gas and isotopic mixture of close molecular weights be used to avoid slow molecular diffusion⁶. In the present method, because of the inverse relationship between axial dispersion and molecular diffusion (equation (1)), a heavy carrier gas produces a fast dispersion which results also in a higher separation factor. It can then approach the limiting value of $1 + 1.12(m_2/m_1)^{1/2}$.

We have represented infinitesimally small isotopic injections by Dirac functions, in order to get analytical expressions for the separation factor. But, if the mean fractional concentration of the injected mixture in the principal tube is equal to 0.1, numerical integration shows that the separation factor should be multiplied by 0.965.

In Table 1 we present separation factors for isotopes $C^{12}-C^{13}$ given by Benedict and Pigford⁶ for different methods of isotopic separation as well as for the present one. These values are theoretical and can be much smaller in practice. The experimental factor for gaseous diffusion, for example, is about half the theoretical one. Our experimental evidence is sufficiently conclusive for us to believe that in the present method, the experimental separation factor will be much closer to the theoretical one. Indeed, our experiments have shown¹⁰ that the concentration curves, which are not disturbed by the movement of valves, fit equation (3) very well.

Table 1 Comparison of α for separation of C^{12} and C^{13} using different methods

Separation method	Working substances	α
Chemical exchange	HCN-CN ⁻	1.013
Distillation	CO	1.010
Gaseous diffusion	CH ₄	1.030
Mass diffusion	CH ₄ -H ₂ O	1.016
Thermal diffusion	CH ₄	1.0026
Gas centrifuge	CH ₄	1.009
Present method (with the mean isotope concentration equal to 10%)	CH ₄ -C ₄ F ₁₀	1.031

The separation of stable light isotopes constitutes probably the most interesting short-term application of our method. The energy cost of separation constitutes only a few per cent of the present cost of stable isotopes. The possible application for enriching uranium in isotope 235 is related to the availability of very heavy molecules. Using $N(C_4F_9)_3$ as carrier fluid, this would lead to a separation factor of 1.0031. The construction of an experimental device for separating uranium isotopes is now under study.

I thank Professors A. Jaumotte and J. E. Dumont, Drs J. W. Turner and I. Paiva-Veretennicoff for help and advice, Professor J. Jeener for stimulating discussions, Mrs D. Leemans for typing the manuscript and Mr and Mrs Pengally for revising the English text. This work was supported by the Conseil National de la Politique Scientifique.

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Received 13 September; accepted 9 November 1977.

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First proof of structure of a C_{28} -pentacyclic triterpane in petroleum

DURING the past decade, C_{27} , C_{29} , C_{30} , and C_{31} modified pentacyclic triterpane hydrocarbons, mainly hopanes, have been discovered in fossil fuels¹⁻⁴. Besides their fundamental importance as diagenetic products from the conversion of living organisms to hydrocarbon deposits in nature, the interpretation of their structure in terms of precursor-product relationships promises to be useful for problems of petroleum exploration^{5,6}. The reported presence of 'degraded triterpanes containing 27 and 29 but not 28 carbon atoms'² has mechanistically been rationalised² in analogy to the observed absence of C_{17} -isoprenoid hydrocarbons in fossil fuels because of the unlikelyhood of cleavage of two carbon-carbon bonds located at the same carbon atom (C-22) of the hopane side chain. We determine here the structure of a C_{28} member of the hopane family, 17 α (H), 18 α (H), 21 β (H)-28,30-bisnorhopane (Fig. 1) and its enormous natural abundance (26 p.p.m. of rock) in the Monterey shale, offshore Santa Barbara, California.

After isolation of saturates⁷ from the shale extract, a concentrate containing 50% of C_{28} -triterpane was isolated by preparative gas chromatography⁷. Further purification on acidic alumina I (ref. 8) revealed the presence of at least two components in the ratio of 3 : 1 and led to the isolation of $C_{28}H_{48}$ -triterpane-I (Fig. 1), the predominating component, in crystalline form (melting point 150–152 °C), by slow evaporation of a concentrated hexane solution.

Proof of structure of $C_{28}H_{48}$ -triterpane-I as being a bisnorhopane with methyl groups missing at C-18 and C-22 and 17 α (H), 21 β (H) stereochemistry, being 17 α (H)-28-noradientane, was obtained as follows: (1) mass spectrometry: 70 eV, m/e (relative intensity) 384 (95, M⁺), 369 (12, M-CH₃), 355 (10, M-C₂H₅), 191 (100, rings A+B), 177 (20), 163 (30, rings D+E), 149 (15). The observed ratio of intensities at m/e 191 to that at 163 is consistent with 17 α (H), 21 β (H) stereochemistry³. The m/e 355 fragment signals an intact ethyl side chain. The remaining methyl deficiency must, therefore, be one of the angular methyl groups on the triterpane nucleus. The high

abundance of m/e 191 indicates the A and B portion of the hopane ring system intact. The facile cleavage of C-8/C-14 bond to produce the two major fragments at m/e 191 and 163 indicates the presence of 8 β - and 14 α -methyl groups. Thus, the methyl group must have been lost from C-18. The mass spectral evidence by itself, however, does not permit clear distinction from an alternate structure, namely, 28,30-bisnorlupane. Elimination of the latter and confirmation of the structure of $C_{28}H_{48}$ -triterpane-I (Fig. 1) was achieved by X-ray crystallography.

(2) X-ray crystallography: the compound is orthorhombic, space group P2₁2₁2₁, with $Z = 4$. The cell parameters are: $a = 11.26$ Å, $b = 7.36$ Å, $c = 28.38$ Å. 3,090 Reflections were measured on an automatic diffractometer of which 2,242 were considered to be observed. The structure was solved by direct methods, and the data were refined to $R = 0.068$ by block-diagonal least-squares refinement of structure factors. The mean bond distance over 32 values is 1.536 (21) Å; and all distances are within the 2 σ limit except the C-8/C-14 distance, which is 1.600 Å, analogous to 1.622 Å in 17 α (H)-adientane¹⁰, explaining the observed facile cleavage of this bond by mass spectrometry.

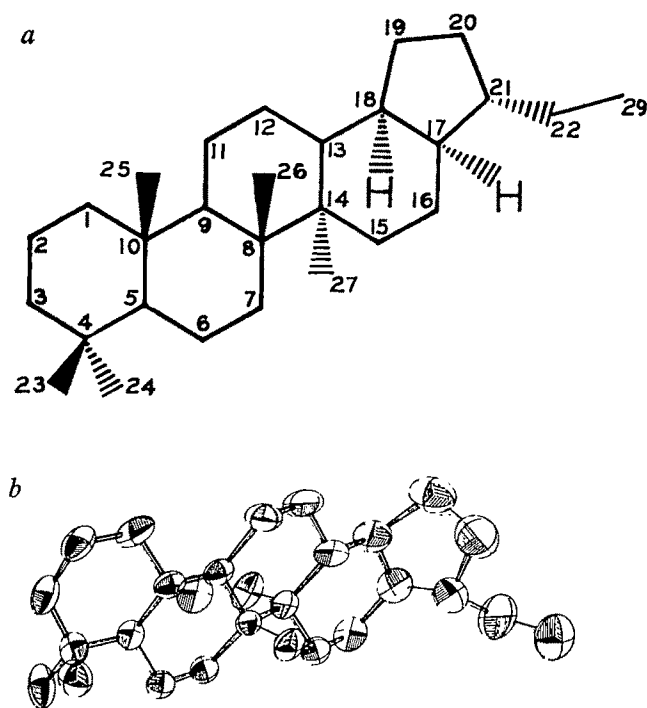
(3) NMR: 360 MHz, (CDCl₃) δ 0.793 (3,s,4 β -CH₃), 0.834 (3,s,4 α -CH₃), 0.843 (3,s,10 β -CH₃), 0.886 (3,t, $\delta = 7$ Hz, 22-CH₃), 0.899 (3,s,14 α -CH₃), 0.937 (3,s,8 β -CH₃); the chemical shifts of the 4 β , 4 α , and 10 β -methyl group protons are equivalent to those shifts for 17 α (H)-hopane [(CDCl₃) δ 0.80 (4 β), 0.83 (4 α), 0.85 (10 β)] (ref. 11), and the 8 β -methyl protons are only slightly shifted from those of 17 α (H)-hopane [δ 0.96 (8 β)] (ref. 11) confirming the natural carbon skeleton for the ring A-B portion of $C_{28}H_{48}$ -triterpane-I. The relatively large upfield shift for the 14 α -methyl protons from 1.00 p.p.m. in 17 α (H)-hopane¹¹ to 0.899 p.p.m. in $C_{28}H_{48}$ -triterpane-I is consistent with a relief of steric interaction by removal of the 18 α -methyl group, whose signal is absent in $C_{28}H_{48}$ -triterpane-I [0.91 p.p.m. in 17 α (H)-hopane]¹¹.

The particular C_{28} -triterpane reported here seems to be either absent, or present only in traces, in most fossil fuel samples¹⁻⁴. However, it is present in many California crudes⁵ and occurs with abundance in the nearby Monterey shale and presumably also in large abundance in a crude form from the Northern Volga Ural (Siva) Region (USSR)¹². This points toward some unique diagenetic pathway. Present speculation centres on adipedatol¹³, a fern triterpenoid of the 30-norhopane type possessing a hemiacetal linkage between C-22 and C-28. Hydrolysis would yield a CH₂OH-group at C-18 whose oxidation to the corresponding carboxylic acid and subsequent decarboxylation, all accepted geochemical processes¹⁴, would yield the identified hydrocarbon (Fig. 1).

An alternate precursor-product relationship can be visualised starting with adiantone (C_{29}). The latter was postulated¹⁵ to be the precursor for certain partially aromatised hopanes in fossil fuels of proven structure possessing the ethyl side chain in ring E, analogous to $C_{28}H_{48}$ -triterpane-I. The progressive aromatisation required is assumed to begin in ring D. Thus, introduction of the first double bond by reduction of adiantone, dehydration, and shift of the double bond into ring D into the most stable 13(18) position¹⁶ under strongly acidic conditions must result in elimination of the methyl group at C-18. Hydrogenation at this point, parallel or in lieu of aromatisation due to the particular geochemical environment, would yield our $C_{28}H_{48}$ -triterpane-I. Similarly 21-hydroxyadientone¹⁷, a fern constituent, could give rise to our C_{28} -triterpane through formation of a conjugated diene, accompanied by loss of the methyl group at C-18 and subsequent hydrogenation.

Finally, to explain the unusual abundance of this bisnorhopane in selected cases (Monterey, Siva), there is the remote possibility of a molecular sieve effect in nature, that is enrichment by molecular size due to pore size of the particular inorganic matrix present. Current investigations deal with the structure of the second $C_{28}H_{48}$ -triterpane isomer present in minor quantity.

Fig. 1 Structure of $C_{28}H_{48}$ -triterpane-I. a, 17 α (H), 18 α (H), 21 β (H)-28,30-bisnorhopane; b, stereoscopic view drawn by ORTEP⁹, vibration ellipsoids scaled to 50% probability.



We thank Professor Carl Djerassi and Dr Lois Durham for use of the HXS-360 in the Stanford Magnetic Resonance Laboratory which is supported by NSF grant no. GR 23633 and NIH grant no. RR00711, also we thank the management of Chevron Oil Field Research, La Habra, California, and BP Co., Sunbury, for permission to publish.

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Received 21 October; accepted 1 December 1977.

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Strontium isotope evidence for petrogenesis of Mexican andesites

THE Mexican volcanic belt (MVB) consists of several inactive and active volcanoes¹ (Fig. 1). The active volcanoes include Colima^{1,2} and Ceboruco³ near the Pacific coast, Popocatepetl and Iztaccihuatl in central Mexico, and San Martin⁴ close to the Caribbean coast. The MVB rests on continental crust approximately 40 km thick^{5,6}, and is associated with subduction of the Cocos plate below central Mexico^{7,8}. The link between plate subduction and the disposition of the MVB is not simple since the latter makes an angle of about 20° with the Middle America Trench⁹. This complexity might be related to the structure of the continental crust⁵, or to a combination of subduction and extensional processes⁴. The most prominent products of volcanism of the MVB are the large composite volcanoes built up from successive eruptions of intermediate lavas and pyroclastic rocks^{3,8}. The lavas of the active volcanoes shown in Fig. 1 are dominantly calc-alkaline andesite and dacite^{3,8–10}. In contrast, the easternmost active volcano, San Martin, is built from lavas of a picritic basalt-basanitoid-alkali basalt-hawaiite association^{4,11,12}. This volcanic area occurs at a change in direction of the MVB and might therefore be linked with extension or fracturing associated with the destructive margin setting of the MVB (ref. 4). Here we report new Sr isotope data for lavas from Ceboruco, Colima, and San Martin. These data, and published Sr isotope data for Cainozoic volcanics from central Mexico¹³ constitute evidence for petrogenesis of Mexican volcanic rocks.

The only published Sr isotope data for Mexican lavas are from rocks of Miocene–Recent age from the area

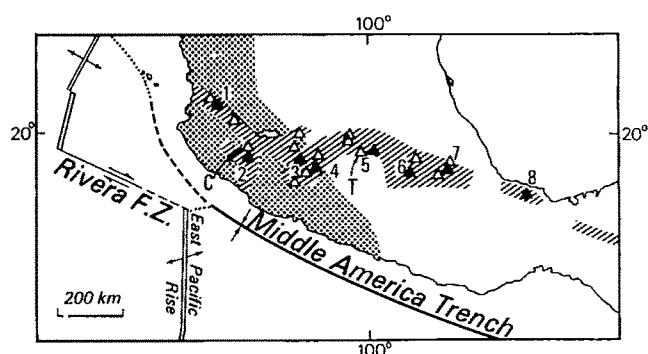


Fig. 1 Volcanoes and plate tectonics of Central Mexico. The two Cainozoic volcanic provinces are shown: the stippled area is the Cordilleran Province and the diagonal shading is the Mexican volcanic belt³. Δ , Inactive composite volcanoes including C, Nevado de Colima and T, Nevado de Toluca. \blacktriangle , Recently active volcanoes numbered: 1, Ceboruco; 2, Colima; 3, Paricutin; 4, Jorullo; 5, Xitli; 6, Popocatepetl; 7, Orizaba; 8, San Martin.

around the inactive volcano, Nevado de Toluca^{13,14} (Fig. 1). Whitford and Bloomfield¹³ have obtained Sr isotope ratios in the range 0.7032–0.7045 for 30 samples of lavas from the Toluca area. There is no overall correlation between $^{87}\text{Rb}/^{86}\text{Sr}$ and $^{87}\text{Sr}/^{86}\text{Sr}$, but 10 samples from the immediate vicinity of the volcano show a significant positive correlation indicating an apparent age of 430 ± 97 Myr (ref. 13, p. 211). Whitford and Bloomfield argue that the relatively low $^{87}\text{Sr}/^{86}\text{Sr}$ ratios suggest an ultimate origin in the mantle (ref. 13, p. 212).

We have determined Sr isotope data for 16 Pliocene–Recent lavas from Ceboruco, the Colima area and San Martin. Sr isotope ratios were determined on a VG-Micromass 30 mass spectrometer using chemical and mass-spectrometric techniques described elsewhere¹⁵. Measurements of the Eimer and Amend standard SrCO_3 carried out during this work yield an overall mean of 0.70814 ± 1 . Rb/Sr isotope ratios were determined by a precise X-ray fluorescence technique¹⁶. The Sr isotope ratios and related analytical data for the Mexican lavas are presented in Table 1. Because the analysed samples are all geologically very young (< 1 Myr), the $^{87}\text{Sr}/^{86}\text{Sr}$ ratios presented in Table 1 are taken as initial ratios. These data are shown as a plot of $^{87}\text{Rb}/^{86}\text{Sr}$ against $^{87}\text{Sr}/^{86}\text{Sr}$ in Fig. 2.

Figure 2 shows that the Sr isotope compositions of samples from the Colima area and from San Martin show slight but significant internal variation within the range

Fig. 2 Plot of $^{87}\text{Sr}/^{86}\text{Sr}$ against $^{87}\text{Rb}/^{86}\text{Sr}$ for volcanic rocks from Mexico. \bullet , Miocene–Recent lavas from the Nevado de Toluca area¹³; \circ , from Colima and Nevado de Colima; \square , from Ceboruco; \triangle , from San Martin (see Table 1).

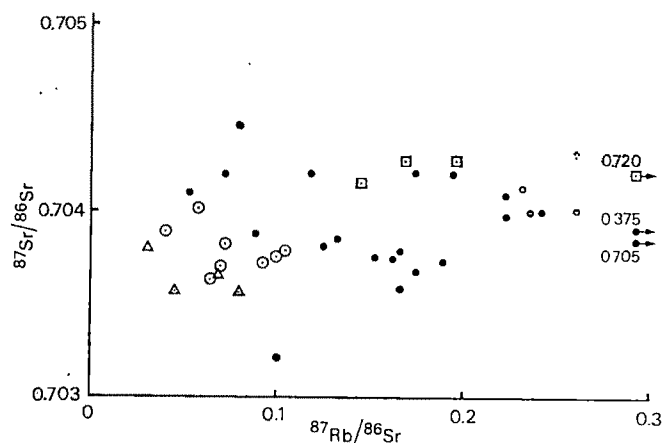


Table 1 Analyses of Mexican lavas

Specimen no.	Rock type	Rb* (p.p.m.)	Sr* (p.p.m.)	⁸⁷ Rb/ ⁸⁶ Sr†	⁸⁷ Sr/ ⁸⁶ Sr‡
Nevado De Colima					
C18	Andesite	19	785	0.072	0.70384 ± 6
C20	Andesite	19	871	0.064	0.70363 ± 6
C27	Andesite	21	648	0.093	0.70373 ± 7
Colima					
C39	Basaltic andesite	24	1,750	0.041	0.70388 ± 5
C40	Basaltic andesite	38	1,932	0.058	0.70402 ± 5
C36	Andesite	20	564	0.104	0.70378 ± 7
C37	Andesite	20	565	0.101	0.70376 ± 7
C109	Andesite	13	557	0.069	0.70369 ± 7
Ceboruco ^a					
M43	Andesite	35	527	0.194	0.70427 ± 5
M45	Andesite	35	586	0.171	0.70427 ± 6
M46	Andesite	31	628	0.145	0.70414 ± 5
M42	Dacite	61	246	0.720	0.70420 ± 7
San Martin ^a					
M71	Picritic basalt	14	1,312	0.029	0.70380 ± 6
M76	Basanitoid	15	663	0.067	0.70366 ± 8
M63	Alkali basalt	19	679	0.081	0.70358 ± 6
M74	Alkali basalt	10	660	0.044	0.70357 ± 6

* Determined by X-ray fluorescence. Mass absorption coefficients estimated from Compton scattering, concentration data about ± 5%.

† Average precision ± 1% (for XRF-techniques, see ref. 16).

‡ Within-run precision quoted as 2 × s.e.m.

0.7036–0.7040 but lie entirely within the range of data for the Toluca area reported by Whitford and Bloomfield (0.7032–0.7045, ref. 13). In contrast, the Ceboruco samples show virtually no significant variation and have ⁸⁷Sr/⁸⁶Sr ratios higher than any data from Colima or San Martin with an average of 0.7042. None of the new sets of data for individual volcanoes show significant correlation of ⁸⁷Sr/⁸⁶Sr with Rb⁸⁷/Sr⁸⁶ (see ref. 17). These data enable us to comment as follows on the petrogenesis of Mexican volcanics.

Values of ⁸⁷Sr/⁸⁶Sr for all analyses of andesitic volcanics fall into the range reported for intermediate lavas from Central America^{18,19}, and for lavas from volcanic arcs where sialic crust is absent^{20–22}. The low Sr isotope ratios for these andesites, commonly in the range 0.7035–0.7040, suggest that sialic contribution to their origin is insignificant.

The mean Sr isotope ratio of 0.7042 of the Ceboruco lavas is significantly higher than that for most of the other samples plotted in Fig. 2, although slightly lower than most continental andesites from South America^{23,24}. These higher ratios could be accounted for by slight contamination of Ceboruco lavas with crustal Sr (compare ref. 23). Ceboruco is also anomalous in showing a high rate of increase in K₂O relative to SiO₂ in comparison with other volcanics from the MVB³. In view of the internal consistency of the Ceboruco Sr isotope ratios, and the similarity of absolute Sr concentrations in Ceboruco lavas to other Mexican andesites (Table 1), it is possible that the higher Sr isotope ratios reflect the Sr isotope composition of the mantle below the volcano. The Sr isotope data therefore suggest a small degree of mantle Sr isotope heterogeneity below the western part of the MVB.

The Sr isotope ratios of the basanites and alkali basalts from San Martin fall into the general range reported for alkali basalts from elsewhere^{25–27}. We suggest that the similarity of Sr isotope values for San Martin basalts with most Mexican andesites indicates a common mantle source.

The Sr isotope data are furthermore consistent with experimental evidence that undersaturated basalt and over-saturated andesite might both be contrasted products of partial melting of mantle peridotite under different conditions of pressure, temperature and activity of water in the coexisting volatile phase²⁸.

Rock samples were collected from Colima during a visit sponsored by Earthwatch (Educational Expeditions International) and the Centre for Field Research (Belmont, Massachusetts). We thank the expedition participants for help in collecting samples and J. S. Vizcaino for much help on Colima. The isotope work at Oxford is supported by NERC.

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Rapakivi granite, anorthosite and charnockitic plutonism

THE close association of massive anorthosite and charnockitic granitic rocks is well documented¹. Rare earth element (REE) investigations²⁻⁴ have indicated that the massive charnockite (mangerite) associated with anorthosite is not comagmatic with it but represents a distinct magma fraction. Bridgwater *et al.*⁵ describe a charnockitic mantle around a plutonic suite of norites, monzonites and rapakivi granites from the Kap Farvel district of south Greenland which they consider to represent a parallel situation to the anorthosite-mangerite association. They believe contact metamorphic processes accompanied by anatexis produced the charnockitic phases. The charnockites are, therefore, dependent on the associated igneous suites but are not comagmatic with them. Duchesne *et al.*⁷ have investigated the REE content of monzonitic rocks considered consanguineous with the anorthosites of the South Rogaland district of southern Norway. The absence of any Eu anomaly in the monzonitic rocks, as compared with the marked positive Eu anomaly found for the anorthosites, places severe constraints on any postulated common parent magma for this rock association. A magma produced by partial fusion of upper mantle Kaersutite has been suggested⁷. A link between anorthosite and rapakivi granite⁸ has been proposed, rapakivi granite being a high level crystallisation related to and contemporaneous with anorthosite emplacement at greater depth. From their study of the Pikes Peak Batholith, Colorado, Barker *et al.*⁸ proposed a composite model for the origin of the rock spectrum gabbro-anorthosite-syenite-granite (including rapakivi granite). Convecting, mantle-derived, alkaline olivine basalt magma is believed to produce a quartz-syenitic magma from K₂O-poor lower crustal rocks which in turn reacts with the granodioritic to granitic rocks of higher crustal levels to produce biotite and biotite-hornblende granites. Anorthosite is a precipitate phase from intermediate liquids. Our results from an REE investigation of plutonic charnockitic rocks and associated granites from south-west Sweden suggest that rapakivi granite may be associated with the charnockites satellitic to anorthosite rather than with anorthosite itself.

The southern parts of the south-west Swedish gneiss belt have much in common with the south Norwegian anorthosite province although no massive anorthosite has been located in the Swedish area. An intrusive, compound, plutonic association, the Charnokite-Granite Association (C.G.A.), which includes both charnockitic and non-charnockitic components, outcrops in the Varberg area of south-west Sweden⁹. This complex was initiated, mobilised and emplaced during a metamorphism which produced *in situ* charnockitisation within the country rock sequences; meta-arkosic gneisses and a more varied layered sequence with a significant basic metavolcanic com-

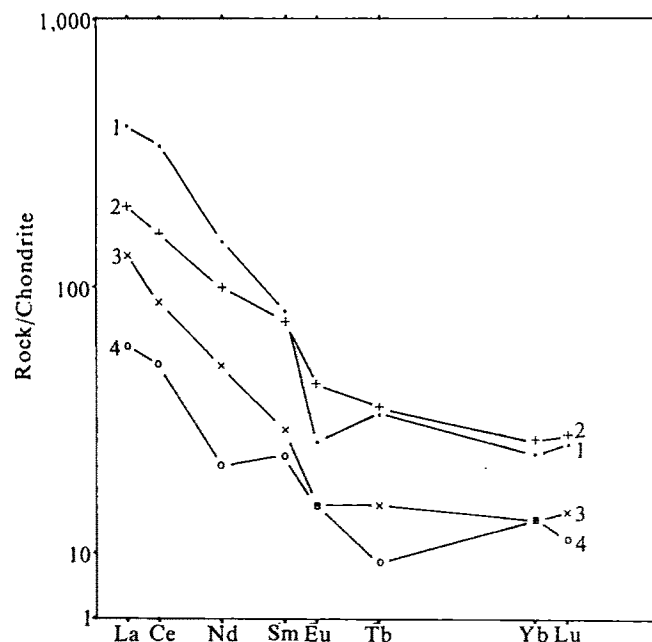


Fig. 1 Chondrite-normalised REE distribution curves. 1. Torpa granite of the C.G.A. 2. Charnockitic members of the C.G.A. 3. Charnockitised granite gneiss, Varberg Formation. 4. Charnokitic quartzofeldspathic granulite, Varberg Formation. Data of Table 1 normalised against Leedey L6 chondrite.

ponent. The admixture of charnockitic and non-charnockitic elements in the C.G.A. is interpreted on the basis of an internal differentiation during emplacement, controlled by H₂O aggregation. The ultimate result of the segregation process was the separation of a differentially mobile, relatively 'wet', granite fraction from a comparatively sluggish, 'dry', intermediate residuum. The latter crystallised as massive charnockite without having travelled far from the site of original anatexic melt generation. By virtue of its differing physicochemical properties, however, the granite differentiate was able to rise further and could separate from its cogenetic charnockite associates. In the Varberg occurrence, however, the link with the charnockites was never completely severed. The advancing head of the granite (the Torpa granite) passed from the granulite sequence, thought to represent reworked basement, to an overlying sequence of layered metasedimentary cover rocks where it spread laterally to crystallise as a series of subconformable sheets⁹. This effectively halted further separation.

The rare earths La, Ce, Nd, Sm, Eu, Tb, Yb and Lu have been determined by instrumental neutron activation analysis for the charnockitic and non-charnockitic elements of the C.G.A. and quartzofeldspathic components of the country rocks (Table 1). The chondrite-normalised averaged distribution curves are presented in Fig. 1. If the C.G.A. is an anatexic derivative from rocks similar to its country rocks, as is believed, a significant accumulation of rare earth elements is involved. All rock groupings show strong differentiation, generally with fairly marked inflexion. The C.G.A. charnockitic rock pattern is similar to that obtained for the Quebec mangerite by Philpotts *et al.* but differs from that found for the massive mangerite of the Lofoten-Vesterdaalen province by Green *et al.*⁴ in that there is no sign of the development of a positive Eu anomaly. It is, however, similar to the pattern for the Moskensøy gneisses marginal to the Lofoten anorthosite⁴. The change from charnockite to non-charnockite within the C.G.A. is marked by concentration of the light rare earth elements La, Ce and Nd, increase in total REE and increase in the differentiation index La/Yb from 12 to 27. The non-charnockitic Torpa granite is further distinguished by the development of a negative Eu anomaly.

In Fig. 2 the chondrite-normalised REE distribution plot for the Torpa granite is compared with some average granite

Table 1 REE concentrations in charnockitic and non-charnockitic quartzofeldspathic rocks from the Varberg area (in p.p.m.)

	La	Ce	Nd	Sm	Eu	Tb	Yb	Lu
1	152	334	106	17.0	3.7	2.0	5.7	0.9
2	77.2	155	70.3	18.4	3.0	1.9	6.5	1.0
3	49.7	85.6	36.3	6.8	1.3	0.9	3.3	0.5
4	22.7	49.5	15.0	5.1	1.3	0.5	3.3	0.4

1. Torpa granite (non-charnockitic) of the C.G.A. (three analyses).
 2. Charnockitic phases of C.G.A. (nine analyses).
 3. Granite-gneiss (meta-arkose) (four analyses).
 4. Quartzofeldspathic granulite (two analyses).

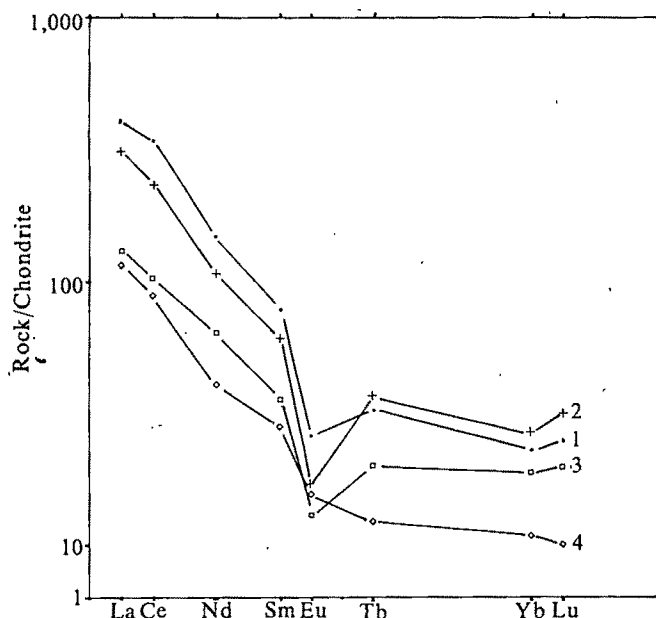


Fig. 2 Comparison of granitic REE chondrite-normalised distribution plots. 1. Torpa granite of C.G.A. 2. Finnish rapakivi granites (after Koljonen *et al.*⁸). 3. 70% SiO₂ granite composite (after Haskin *et al.*⁹). 4. Svecofennian granite (after Koljonen *et al.*⁸).

plots^{10,11}. In both total REE and differentiation style the Torpa granite is strikingly similar to the Finnish rapakivi granites, as reported by Koljonen and Rosenberg¹⁰ and Vormia¹². Identity is marred only by the more pronounced Eu anomaly shown by the Finnish rocks. Both the Swedish and Finnish rocks have REE chemistries which distinguish them from granites of comparable major element composition.

The similar and distinctive REE characteristics of the Finnish rapakivi granites and the Torpa granite of south-west Sweden suggest that they may be similar rocks with a comparable petrogenesis, that is, the Torpa granite is of Finnish rapakivi type. The distinctive mantled megacryst feldspar rapakivi texture occurs, albeit sparingly, within the Torpa granite mass. Although variation in major element chemistry is considerable within rocks recognised as belonging to the rapakivi suite, the SiO₂ range is 64–72%, K₂O is high and MgO always very low⁸. The average SiO₂ for the Torpa granite is 70.7%, K₂O is 5.58% and MgO is 0.52%. The corresponding figures for a rapakivi granite from Satakunta, Finland¹⁰, are: SiO₂ = 68.16%, K₂O = 6.89% and MgO = 0.50%. In contrast, the average values for the charnockites of the C.G.A. are: SiO₂ = 62.73%, K₂O = 4.61% and MgO = 1.51%.

In the Varberg occurrence the relationship of the rapakivi type Torpa granite to the charnockitic plutonism is direct and clearly observable in the field. It is possible that a similar relationship is preserved in the border zones of the large Wiborg rapakivi pluton of south Finland where the red rapakivi granite is reported to grade to dark green rocks akin to mangerite found associated with Precambrian anorthosites⁸. In many cases, however, the granite may have separated entirely from the charnockitic source areas.

On the basis of this study it is proposed that, if a connection exists between rapakivi granite and massive anorthosite, it is indirect. The emplacement of anorthosite in a high-grade crustal environment induces charnockitic recrystallisation and anatectic plutonism in the adjacent country rocks. In some cases, the products of this plutonic activity may differentiate to produce a mobile granitic fraction of rapakivi type which may rise to crystallise at higher crustal levels, sometimes completely dissociated from its source. Rapakivi granite is a product of charnockitic plutonism which in some cases is triggered by anorthositic emplacement.

In the Varberg area no anorthosite is exposed but may exist at unexposed levels. The accession of hot mantle material other

than anorthosite to the deeper crustal levels might be expected to engender charnockite-rapakivi plutonism in a similar manner. There is evidence in the south-west Swedish province of significant ultrabasic and basic magmatic activity contemporaneous with the charnockitisation.

We thank the Carnegie Trust for the Universities of Scotland for financial aid.

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Received 30 August; accepted 30 November 1977.

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Apomixis may be widespread among trees of the climax rain forest

THE exceptional species diversity of tropical rain forests is well known; of these the lowland forests of the Malay Peninsula are among the richest¹. This, and the size of the trees, pose obvious difficulties for the maintenance of panmixis, and tempt speculation on the processes by which such diversity has evolved and may now be favoured. Fedorov² suggested that natural selection is low and self-pollination prevalent, favouring random genetic drift. Prevalence of random drift, or apomixis—agamosperry—would contradict Ashton's³ view that, given a stable environment, evolution proceeds through ecotypic differentiation while diversity accrues through ever increasing niche specialisation; for this view to be correct, maintenance of genetic variability within breeding groups would be essential. Solid evidence has been meagre but there is evidence⁴ that genetic polymorphism, and hence heterozygosity, is common in the emergent tree *Shorea leprosula* Miq., and *Xerospermum intermedium* Radlk., an understorey species. The pattern of spatial variation is consistent with the view that these species are outbreeders in which predominantly short-range pollen and fruit dispersal are accompanied by short-range heterogeneity in gene frequencies. However, both are common trees with minimal spatial isolation. We now report the occurrence of apomixis in some other rain forest trees.

Apomixis through adventive polyembryony is well known in cultivated mangoes⁵ whose wild relatives are main canopy trees of the mature phase rain forest, and in cultivated *Citrus*, *Eugenia*, *Garcinia mangostana* Roxb. and *Lansium*^{6–8}, fruit trees which originated from its understorey. We do not know whether such patterns of embryogenesis have arisen after cultivation. Apomixis can also be inferred from triploidy in root-tip squashes derived from several seedlings originating from a single tree of the dipterocarp *Hopea latifolia* Sym. ($2n = 3x = 21$) (misidentified at the time as *H. beccariana*) in Semengoh Forest, Sarawak, West Borneo⁹. A.K. found triploidy also in *Hopea subulata* Sym. ($2n = 3x = 21$), where she also observed that some, or in certain cases all, the embryo sacs abort well before anthesis.

Apomixis can now be demonstrated in the Malayan

emergent dipterocarp *Shorea ovalis* (Korth.) Bl. ssp. *sericea* (Dyer) Ashton, in which, as noted previously, experimental selfings and crossings each lead to similar and relatively high levels of fruit set, and in *S. agami* Wood ex Ashton. *S. leprosula*, with which they grow in mixture, is a diploid with a high level of self-incompatibility in which embryo sac development is of the *Polygonum* type, and embryogenesis is normal. In *S. ovalis* we found that a suite of at least five proembryos develops at the micropylar end from the nucellus; these are therefore almost certainly genetically identical to the parent. Frequently only two seedlings germinate from a single seed and this suggests that only a small proportion of proembryos reach maturity. *S. agami* manifests essentially the same pattern although fewer proembryos seem to be formed. *S. ovalis* examined by us are tetraploid ($2n = 4x = 28$), with meiotic irregularities in the form of univalent and multivalent chromosome associations^{9,10}. The low level of genetic polymorphism reported earlier in this species⁴ may thus be an artefact occasioned by polyploidy.

Table 1 shows that at least one of a syndrome of characters often associated with apomixis, including the occurrence of multiple seedlings in normally single-seeded fruit, high levels of putative self-compatibility, low pollen germinability, irregular meiosis and polyploidy, may occur in rain forest dipterocarps of both canopy and understorey.

Maury¹¹ observed multiple seedlings in, among others, two close relatives of *S. leprosula*: *S. parvifolia* Dyer ($2x = 14$), in which we failed to find any multiple seedlings in

more than 300 seeds examined from another population and *S. argentifolia* Sym. ex Wood ($2x = 14$), in which we found a low percentage. She believed, though on inferential evidence only, that *H. odorata* and possibly also sometimes *S. parvifolia*, *S. ovalis*, *S. argentifolia* and *S. macrophylla* can also exhibit true polyembryony.

The dipterocarp fruit is trilobular and each loculus as a rule has two ovules. Foxworthy¹² noted long ago that several seedlings habitually germinate from fruit of *S. resinosa* Foxw., and we have observed as many as 18; from our counts (several seedlings from one tree) this species is triploid ($2n = 3x = 21$). Other dipterocarps in which multiple seedlings have been recorded include *Dipterocarpus alatus* Roxb., *D. tuberculatus* Roxb. and *Shorea robusta* Gaertn. f., all of which are gregarious species of the Asian seasonal tropics¹², and the semigregarious African savanna dipterocarps *Monotes kerstingii* Gilg, *M. ? elegans* Gilg and *M. madagascarensis* Humb. (subfamily Monotoideae)¹³. Among 16 Malayan dipterocarp species studied quantitatively by us at least 10 sometimes produce multiple seedlings. It is yet to be confirmed whether in these apomixis is associated with production of multiple seedlings other than in the cases mentioned here; we assume from studies to be published elsewhere that at least in *S. resinosa* and *S. macrophylla*, and it is possible, of course, that adventive embryony occurs in seeds of these and other species in which single embryos develop as well. The percentage of seeds yielding multiple seedlings was estimated in several individuals of *S. agami*, *S. ovalis*, *S. parvifolia* and *S. macro-*

Table 1 Reproductive characteristics of some Malayan Dipterocarpaceae as evidence for apomixis

Species	Chromosome no.	Putative self-compatibility	Pollen germinability	% Seed with multiple seedlings*	Embryological evidence for apomixis	Understorey (U), main canopy (C) or emergent (E)
Evidence not available or negative						
<i>Shorea acuminata</i> Dyer	$2x = 14$			0		E
<i>lepidota</i> Bl.				0	Negative	E
<i>leprosula</i> Miq.	$2x = 14$	Low	High	0	Negative	E
<i>multiflora</i> (Burck) Sym.				0		C
<i>seminis</i> (de Vr.) Sloot.				0		E
<i>stenoptera</i> Burck	$2x = 14$			0		U
<i>Dipterocarpus oblongifolius</i> Bl.		High		0	Negative	E
Evidence slight or tentative						
<i>Anisoptera curtisii</i> Dyer ex King				Low†		E
<i>S. argentifolia</i> Sym. ex Wood	$2x = 14$			2 (2)	Tentative†	E
<i>gratissima</i> Dyer				Low†		E
<i>macrophylla</i> (de Vr.) Ashton	$2x = 14$	Low	High	Low† (2†)	Tentative‡	C
<i>ovalis</i> (Korth.) Bl. ssp. <i>ovalis</i>				Low† (2†)		E
<i>parvifolia</i> Dyer	$2x = 14$			Variable, low	Tentative‡	E
<i>pauciflora</i> King	$2x = 14$			2 (3)		E
<i>smithiana</i> Sym.	$x = 7$			4† (2†)		E
<i>Hopea mengarawan</i> Miq.				8† (2†)		C
<i>Dipterocarpus baudii</i> Korth.	$2x = 22$			2† (2†)		E
<i>cornutus</i> Dyer				Low†		E
<i>costulatus</i> Sloot.				2† (2†)		E
<i>Dryobalanops aromatica</i> Gaertn. f.	$2n = 14$			Low††		E
<i>Parashorea densiflora</i> Sloot. et Sym.				Low†		E
<i>Vatica pallida</i> Dyer				Low†		U
<i>pauciflora</i> (Korth.) Bl.				Low†		U
Evidence strong but inferential						
<i>S. macrophylla</i> Dyer ssp. <i>macrophylla</i>	$2x = 14$	Low		30–70, variable(9)	Inferential	E
<i>resinosa</i> Foxw.	$2n = 3x = 21$		Nil	98 (18)	Inferential	E
<i>H. latifolia</i> Sym.	$2n = 3x = 21$				Inferential	C
<i>odorata</i> Roxb.	$2n = 3x = 20–22§$			> 90†	Inferential†	E
<i>subulata</i> Sym.	$2n = 3x = 21$		Nil	21 (3)	Inferential	U
Apomixis demonstrated						
<i>S. agami</i> Wood	$2x = 14$		Nil	> 50, variable(4)	Confirmed	E
<i>ovalis</i> (Korth.) Bl. ssp. <i>sericea</i> (Dyer) Ashton	$2n = 4x = 28¶$	High		15–47, variable(5)	Confirmed	E

*Maximum number per seed is given in parentheses.

†After Foxworthy¹².

‡After Maury¹².

§Unconfirmed.

¶Irregular meiosis in pollen mother cells.

ptera; in each species some trees yielded a high proportion and others few or none.

Maguire¹⁴ has inferred apomixis in *Clusia* (Clusiaceae) from Guyana. One of us (C.O.H.) has obtained successful fruit set on bagged female inflorescences of the wild dioecious understorey fruit tree *Garcinia parvifolia* Miq. of Western Malesia, in the same family. It is not yet confirmed whether embryos develop from the inner integument as in the cultivated mangosteen (*G. mangostana*)⁷. This species has a high, probably polyploid chromosome number ($2n = 60$).

Though it can hardly be claimed that this preliminary evidence adequately represents a tree flora which in the lowlands of Peninsular Malaysia alone runs into thousands, it does conclusively demonstrate the occurrence of apomixis in both emergent and understorey trees of the mature phase. Further, if the close relationship between the small genus *Lansium* and the large *Aglaia* is accepted, all the cases demonstrated are from the series of closely allied species which occur together in the same habitat and which distinguish Malaysian from other tropical forests. Although it is premature to consider these series as themselves evidence of apomictic reproduction comparable to *Hieraceum* or *Rubus*, it is no longer possible to discount the contribution of apomixis to the evolution of some of them and to the high floristic diversity characteristic of lowland tropical rain forest. Western Malesia seems to have had a more uniform climate during the Pleistocene¹⁵ than other continental regions of the humid tropics. If we assume that natural selection in these forests is dominated by biotic factors¹⁶ and that immigration and extinction are continuing, we must conclude that the selective factors are constantly changing, and that few apomictic biotypes will survive for prolonged periods. This suggests that series of allied taxa which include apomicts are evidence for continuing active diversification.

We thank Professor Forbes W. Robertson and J. Muller for helpful suggestions, and Professor A. N. Rao and N. Prakash for guidance in embryology. We thank the Leverhulme Trust Fund, the Royal Society, NERC and the Carnegie Trust for support.

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Regularity versus irregularity in specific songs of closely-related drosophilid flies

THE songs of most insect species are stereotyped and show regular rhythms^{1,2} which can be readily defined and described. In such cases, closely related species produce songs which differ in rhythmic structure³⁻⁵, carrier frequency^{5,6} or combinations of these parameters^{3,7}. We have examined the songs produced during courtship of the sibling drosophilid fly species *Zaprionus tuberculatus*, *Z. sepioides* and *Z. mascariensis* which are known from morphometric⁸ and chromosomal evidence to be closely related. Drosophilid songs are produced by wing beating and normally consist of a regular series of similar mono- or polycyclic sound pulses with carrier frequencies of 150 to 500 Hz and pulse periods of 8 to 250 ms (for review, see ref. 9). Some species produce more than one type of song, and the pulsed song may be interrupted by protracted bursts of constant frequency tone¹⁰.

Flies were lightly etherised on the day of emergence and the two sexes were isolated in groups of 10 to 30 into vials containing fresh standard food: 10-15 d later, one individual of each sex was placed in a 12 × 12 × 20 mm wire gauze cage within 5 mm of the ribbon of a Grampian GR4 microphone (for further information on the acoustics and methods see refs 9, 10). During courtship, flies were watched through a binocular microscope and sounds were monitored through headphones as they were produced. Sound records were made at between 18 and 20 °C using amplifiers onto a Tandberg 3341X tape recorder and subsequently oscillograms were made using a Nihon Kohden PC 2 B moving film camera.

Records were analysed by direct measurement from the oscillograms of pulse period. In addition, the number of pulses making a discrete sequence, separated by at least 1 s from adjacent sequences or by a change of character of 5 pulses duration were measured. Individual sequences were analysed as follows: the mean period and its standard deviation were calculated. From these, variance ratios were obtained and Student's *t* test was applied between the pulse periods in song sequences produced by different individuals of a species and between pulse periods in the songs of two species. In addition, the Kolmogorov-Smirnov test for exponentiality (*D* test)¹¹ was applied to pulse periods in individual sequences of the song of all three species.

Subjectively, the songs of both *Z. tuberculatus* and *Z. sepioides* sounded very regular, with long uninterrupted sequences of regular sound pulses. Analysis showed that each species produced two songs, a short period song which was similar in both species and a long period song of significantly longer period in *Z. sepioides* than in *Z. tuberculatus* (Table 1). In both species, the variability in pulse period within a sequence of song of either type was similar. The type 1 song of *Z. tuberculatus* is produced in longer sequences than that of *Z. sepioides*, but sequence duration is highly variable in both species.

By contrast with the songs of the other two species, that of *Z. mascariensis* always sounded erratic. The duration of a sequence was hard to define and the pulse period within a sequence was highly variable (Table 1). Individual long sequences were tested to find whether the pulse period was random (the *D* test does not discriminate well with short sequences). Of 22 sequences thus analysed, produced by seven individuals, 12 did not differ significantly from randomly produced sequences. All of the other 10 sequences, while showing some pulses produced with a fairly regular short period, also contained

Table 1 Comparison of parameters of songs produced by three species of male *Zaprionus* (Drosophilidae) during courtship at 18–20 °C

Species	Mean pulse period $\pm\sigma$ (ms)	Type 1 song No. of sequences measured	Mean sequence duration $\pm\sigma$ (ms)	Mean no. of pulses per sequence	Mean pulse period $\pm\sigma$ (ms)	No. of sequences measured	Type 2 song Mean sequence dura- tion $\pm\sigma$ (ms)	Mean no. of pulses per sequence $\pm\sigma$
<i>Zaprionus tuberculatus</i> (7)	12.37 \pm 1.32 <i>n</i> = 183	7	730 \pm 531 <i>n</i> = 10	68.3 \pm 50.9 <i>n</i> = 10	26.64 \pm 3.48 <i>n</i> = 240	12	897 \pm 694 <i>n</i> = 10	28.7 \pm 20.7 <i>n</i> = 10
<i>Zaprionus sepsoides</i> (8)	11.23 \pm 1.90 <i>n</i> = 356	14	325 \pm 173 <i>n</i> = 15	29.2 \pm 15.83 <i>n</i> = 15	41.95 \pm 5.21 <i>n</i> = 284	13	1372 \pm 630 <i>n</i> = 15	31.9 \pm 12.96 <i>n</i> = 15
<i>Zaprionus mascariensis</i> (7)	—	—	—	—	81.69 \pm 101.14 <i>n</i> = 880	36	2267 \pm 2162 <i>n</i> = 36	24.9 \pm 21.4 <i>n</i> = 36

Even though the song of *Z. mascariensis* differs from both song types in the other two species, it is treated for the purposes of comparison here as if it were type 2 song. Numbers in parentheses after specific names indicate the individual flies recorded.

long series of erratic pulse periods which were never found in the songs of the other two species. These nonrandom song sequences had a shorter average pulse period and less variability of pulse period than the random sequences (Table 2) but were very significantly more variable than either type of song of the other two species. Although it appears from Table 1 that the duration of song sequences is longer and more variable in *Z. mascariensis* than in the other two species, this is complicated by the fact that in the other species, complex sequences of type 1, then type 2, then type 1, 2–1–2 and several longer combinations have been observed.

It has been shown that the songs of various closely related

which will accept ever more random signals and mechanisms of motor patterning which override the normally rhythmic flight¹⁹ and song-producing mechanisms²⁰.

Irregularity versus regularity as a specific character of insect song has apparently not been previously observed and is thus a rare phenomenon. The situation may be explained as follows: if regularity has evolved, the production and recognition templates will tend to co-evolve and to tighten the song regularity; this is commonly observed. If irregularity once evolves through relaxing of the selective pressure it will tend to proceed to the point of randomness when challenged by nonrandomness; this is what we describe here. Once randomness has evolved, how-

Table 2 Characteristics of song sequence of male *Zaprionus mascariensis*

	No. of sequences observed	Mean pulse period and s.d. (ms)	Mean sequence duration and s.d. (ms)	Mean no. of pulses per sequence and s.d.
Random sequences	12	104.8 \pm 107.2 <i>n</i> = 390	4082 \pm 2685 <i>n</i> = 12	33.6 \pm 22.2 <i>n</i> = 12
Nonrandom sequences	10	53.3 \pm 77.8 <i>n</i> = 405	2319 \pm 1210 <i>n</i> = 10	40.7 \pm 15.2 <i>n</i> = 10
Short sequences	14	111.4 \pm 132.4 <i>n</i> = 85	675 \pm 532 <i>n</i> = 14	6.07 \pm 3.12 <i>n</i> = 14

Short sequences have been taken as those composed of 2–12 pulses which cannot readily be tested for randomness. Nonrandom sequences have a probability of less than 0.05 using the Kolmogorov–Smirnov *D* test¹².

Drosophila species differ in pulse period^{11,13}, and that this difference is an important parameter in courtship success of the male fly and in species isolation^{14,15}. Doubtless such differences are important in distinguishing the rhythmic songs of *Z. tuberculatus* from those of *Z. sepsoides* but it seems that the best character of the song of *Z. mascariensis* by which it can be distinguished from that of the other species is its greater variability and irregular structure. Several advantages have been suggested for the variable song repertoire of birds: that they may be important for individual recognition, in sexual selection or in territorial behaviour (for review see ref. 16). Bird songs, however, do not sound like random sequences, nor are the individual phrases usually as simple as the song pulses of drosophilid flies. Irregularity of the type found in the song of *Z. mascariensis* implies that the female fly has a recognition mechanism capable of accepting irregular sequences and of rejecting regular sequences.

The normal insect situation seems to be that the recognition–production template (for a review of this concept, see ref. 17) is selected to accept ever more stereotyped songs which differ greatly from those of related species from which sexual isolation is desirable¹⁸; this is seen in the type 2 songs *Z. tuberculatus* and *Z. sepsoides* which differ at a level of probability of more than 10^{–6}. By contrast the songs of *Z. mascariensis* which seem to be selected for variability versus regularity will require templates

ever, it is in itself a stable situation from which it is hard to evolve and which therefore is likely to become extinct rapidly and to be rare.

This work was carried out at the Laboratoire de Biologie et Génétique Evolutives, CNRS, 91190 Gif-sur-Yvette, France, as part of a larger and continuing project on the evolutionary biology of drosophilid flies. We pay tribute to the late C. Bocquet, Director of the Laboratory, and thank Drs R. Dawkins, F. H. C. Marriott and J. R. Krebs for advice and assistance, Mme M.-T. Chassagnard for technical assistance and Dr L. Tsacas for initiating the project and for continual support, advice and encouragement. The project was supported by research grant ATP Internationale no. 2164 and RCP no. 318 of CNRS awarded to Dr L. Tsacas.

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Received 2 November; accepted 16 December 1977.

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Semicircular canals in squids

THE cephalopods and fishes are rivals as fast-moving predators and they show many parallel adaptations¹. We present here evidence that in squids and cuttlefishes the statocysts possess semicircular canals, though of rather imperfect form. Animals that move quickly need to monitor angular rotation in order to allow appropriate adjustments of the eyes as they turn. To provide this facility certain critical physical requirements must be met and semicircular canals provide this. Their small size ensures that during rotation the pattern of flow is dominated by viscous damping. Consequently the actual volume of fluid flow is small, permitting "accurate transduction of the volume displacement of fluid by means of a water-tight 'swingdoor' cupula having limited angular excursion in the ampulla"². Furthermore, in vertebrates, the low Reynolds number of the system (less than one) ensures that "the velocity of relative flow becomes strictly proportional to the inertial force driving it and hence to the angular acceleration of the head". The statocysts of the fast-moving cephalopods have gone some way to meet these requirements by their shapes, and by the arrangement of the strange anticristae, projections that partly divide the cavity.

The crista of the statocyst is orientated in several planes³ and is sensitive to both angular and linear acceleration⁴. Further investigation now shows that in squids and cuttlefishes the cavity is shaped to direct the flow of endolymph along specific channels, across which the cupulae of the cristae are placed (Fig. 1). We have studied the canals after fixation and staining

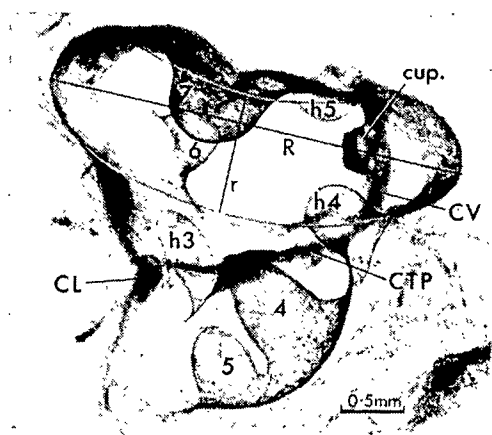


Fig. 1 Horizontal canal of *Loligo vulgaris* of 14 cm mantle length. Left side, seen from behind. The cupula is stained with diluted squid's ink. In all the figures the anticristae and hamuli have been given the numbers used by Dilly, Stephens and Young⁵. CTA and CTP are the anterior and posterior transverse cristae; CL, longitudinal crista; CV, vertical crista; cup., cupula; n.cr., crista nerve; Köf., Kölliker's canal; s., statolith; h1-5 are the hamuli, and the straight anticristae are numbered 1-7.

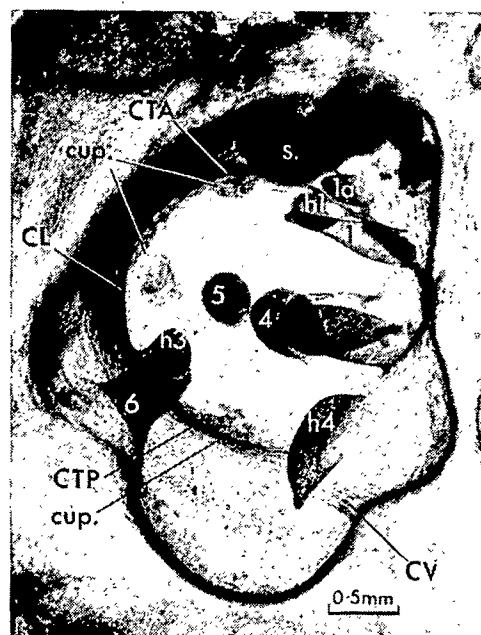


Fig. 2 Left statocyst of *L. vulgaris* stained with haemalum. Seen from above it shows the cupulae on the cristae.

the inside of the statocysts by injection of haemalum or of ink, and by reconstructions from serial sections.

The canals are not complete tubes but are grooves, made by bulges in the outer walls of the sac. The canals are partly enclosed by the projections of cartilage that have been called anticristae³. These projections are of two sorts⁵ (Figs 1 and 2). Some are placed at the turns of the crista, where they bend over as hooks, and these have been called hamuli and numbered h1-h5. Elsewhere the anticristae are straight projections (numbered 1-7) with various shapes, arranged in rows so as to divide the cavity into channels. In an adult *Loligo* some of the anticristae become joined together to make complete tunnels, restricting the flow of endolymph (Fig. 3). Some of the anticristae become shaped and grooved along the lines of flow (Fig. 3).

The most fully developed canal, with a nearly complete tunnel, is the horizontal one, related to the vertical section of the crista and thus monitoring angular accelerations to right or left (yawing). These turns about the vertical axis, produced by the animal's own steering, obviously require corresponding adjustments, especially of the eyes. Movements in the rolling plane, monitored by the longitudinal crista, are smaller and not initiated by the animal itself. The corresponding canal does not have any part that forms a complete tunnel. However, this canal is more complete in *Sepia* than in *Loligo* because in the former there is a large hamulus at the first turn of the crista which is absent in *Loligo*. The anterior transverse crista, along the front of the cavity, monitors movements in the pitching plane and is activated by the endolymph in a deep channel above and below it, but again without a covered tunnel.

The function of the posterior transverse crista is more difficult to decide. It may be activated by movement in the pitching plane, as indicated by the arbitrary lines shown in Fig. 4. Possibly the two transverse cristae serve separately to monitor movement in the two directions, head up and head down, a function that is performed in the other planes by the paired statocysts. However, we suggest that it may (also?) serve to monitor linear accelerations, both forwards and backwards⁶. Squids and cuttlefishes have special need for receptors to monitor these movements produced by their jets. No fish has equal powers of movement in both directions. The channel behind this crista extends far backwards and the slope in front of it is gently downwards. This hypothesis is supported by the fact that the crista faces upwards (Fig. 4) so that its cupula

stands more nearly vertically than those of the anterior transverse and longitudinal cristae, which are horizontal (Fig. 2).

The details of the statocysts vary with the habits of the animals. The anticristae are all more smoothly rounded in *Sepia* than in *Loligo*. *Sepioteuthis* is a squid which resembles *Sepia* in shape, but, of course, is without a cuttlebone. Its anticristae are also rounded, like those of a cuttlefish; perhaps this form is a result of smooth rotatory movements with the fins.

The anticrista at the front of the statocyst (number 1) is rounded and single in *Sepia* but pointed and double in *Loligo* (Fig. 4, numbers 1 and 1a). In the small, inshore, bottom-living squid *Alloteuthis* this anticrista varies greatly between individuals, sometimes forming a plate, sometimes a whole crowd of little knobs. This region lies in the path of movement of endolymph in several planes (Fig. 4) and anticrista 1 is evidently different from all the others. In *Loligo* it is covered with sensory hairs⁵, and perhaps these are receptors serving to monitor acceleration in several planes. *Alloteuthis* has a long rigid spike or 'tail' and this animal may make movements that impose special requirements on the statocyst and produce the varied knobs at the position of anticrista 1.

In cephalopods that turn slowly, such as octopods, or decapods that live in deep waters, the anticristae are reduced^{7,8}. No doubt this is because sensitivity to slow movements requires a large inertial mass of fluid. Conversely, detection of rapid changes in acceleration would be impaired by a large mass, and the rapidly moving decapods avoid this by developing the anticristae and forming partial tubes.

It is interesting to compare the dimensions of the canals of decapods with those of fishes. The radius of curvature (R) of the horizontal canals of four large *Loligo forbesi* gave a mean value of $R = 1.25$ mm. These animals were all around 37 cm in length and weighed about 1 kg (in air). In fishes of this weight the radius was about 5 mm (ref. 9). The internal radius (r) of the channel in the squids, at its narrowest point, was about 0.4 mm, whereas in the fishes it was 0.2 mm. Interpretation using weight for comparison is obviously difficult because of differences in buoyancy. In the cephalopods the small value of R may compensate for the fact that the canals are incomplete and r is

Fig. 3 Thick section of the right statocyst of a large *Loligo forbesi* (48.5 cm mantle length). Seen from the lateral side, to show the fusion of anticristae 2 and 3 and 6 and 7 to make a nearly complete horizontal canal. Anticrista 7 has become elongated along the line of flow. The cupula is very lightly stained and has been touched up. Stained with haemalum.

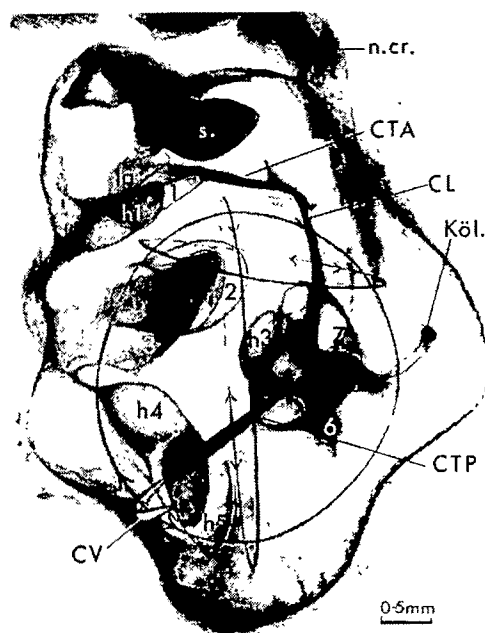
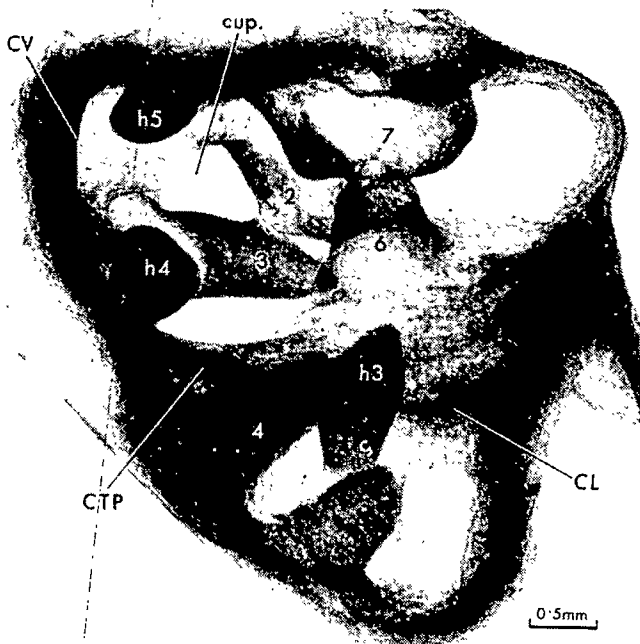


Fig. 4 Right statocyst of a large *L. forbesi* (37 cm mantle length). Seen from above. The presumed directions of flow are shown by arrows. Stained with haemalum. The photograph has been retouched to show the crista more clearly.

large. However, nothing is known of the elastic and other properties of the cupulae, and it is not known whether they allow any flow of fluid past them. Like Budelmann we have seen some appearances suggesting that there may be attachments of the cupulae to the hamuli, perhaps serving to make the channels more nearly 'water-tight'.

It is clear that these devices for canalising flow in the statocyst have the same function as the semicircular canals of vertebrates and the somewhat similar arrangements in Crustacea¹⁰. We must, therefore, add one more to the long list of parallels between the functional organisation of cephalopods and vertebrates¹.

The material for this work was collected at the Marine Biological Laboratory, Plymouth and the Zoological Station, Naples and we thank the Directors and staff of these laboratories for their help. We also thank Dr M. Nixon for help in preparing this paper, Mr C. Carter for photographs, the Wellcome Foundation for support and the Wellcome Institute for the History of Medicine and the Department of Zoology, University College for accommodation and assistance.

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Received 20 September; accepted 9 December 1977.

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Development of eye alignment in cats

WHEN the eyelids of newborn kittens open, casual observation of the pupils suggests that the eyes must be divergently misaligned. Pupillary divergence may be quantified by photographing the eyes while the cat is facing a distant light source. The images of this light, formed by reflection at the corneas, coincide approximately with the optical centres of the eyes, and the distance by which the separation of the pupils exceeds the separation of these images is defined as pupillary divergence¹⁻⁵. It declines with increasing age until some time during the second month². Although it is reasonable to assume that the visual axes are also divergent before this age, this assumption leads to a paradox because surgical misalignment of the eyes of young kittens is known to disrupt binocular connections in striate cortex⁶. Moreover, development of normal orientation selectivity in kitten cortex may require early coordinated binocular vision⁷. We have examined this problem using a combination of techniques with which we are able to determine the alignment of the visual axes in the alert cat. Our results show that while the pupillary axes are quite divergent during the first few weeks after birth, the visual axes are not divergent. It is thus possible that kittens experience synchronised binocular vision very early in life.

The four steps required in the method we use are illustrated in Fig. 1. First, the cat is photographed while alert, as shown in (a). An aperture, positioned in front of a tungsten light source 2.4 metres from the cat, produces a small, well-defined corneal image in each eye. Second, within one day, the cat is prepared for electrophysiological study of single neurones in the visual cortex. The paralysed cat is placed in a stereotaxic

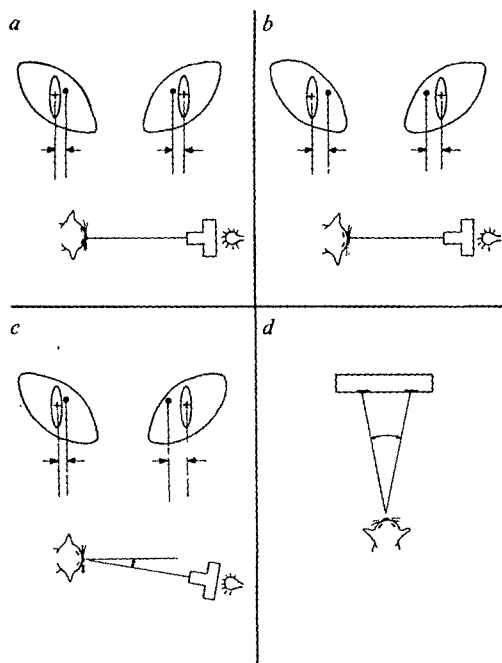


Fig. 1 The steps required to obtain visual axis alignment in the conscious cat. In humans the visual axis or line of sight is defined by a line from the fovea of the retina to the object of fixation passing through the nodal plane of the eye (the nodal plane defines equal magnification between object and image space). In cats we define the visual axis as a line from the centre of the area centralis through the nodal plane to the object of regard. In (a) an alert cat faces a camera and a light source (bottom). The distance by which each pupil centre (+) diverges from the orneal image of the light source (●) is shown (top). The cat is prepared for physiological study and photographs are again taken as shown in (b). To obtain the factor for converting linear to angular divergence, photographs are taken with the camera and light source rotated 10° along an arc (c). Finally, the animal prepared for single-cell study of striate cortex. The angle between the visual axes is then determined by plotting binocular receptive fields as shown in (d).

assembly so that its position is nearly the same as when it was alert and several photographs are again taken, as shown in (b). Third, in order to find the factor for converting linear divergence of the pupils into angular divergence of the pupillary axes, the camera and the light source are shifted 10° along an arc from the previous position, as depicted in (c), and another series of photographs is taken. The fourth step (d) is to plot the receptive fields for several binocular cells recorded from the striate cortex. The mean distance between the centres of these receptive fields is used to determine the angle between the visual axes.

Animals are prepared for physiological study by standard techniques. Anaesthesia is induced with Fluothane, a vein is cannulated, and anaesthesia is maintained with Brevital (sodium methohexital). A tracheal tube is inserted, scalp bone and dura mater are removed, and tungsten-in-glass electrodes are inserted at the crown of the post-lateral gyrus to penetrate in Area 17 within the representation of the area centralis. The animal is paralysed and maintained with a Flaxedil (gallamine triethiodide)-glucose solution. Peak expired CO₂, electrocardiogram and electroencephalogram data are monitored continuously. Spikes from single neurones are amplified and displayed by standard means. Optimal stimuli are determined for each cell and receptive fields are plotted for individual binocular neurones in the visual cortex.

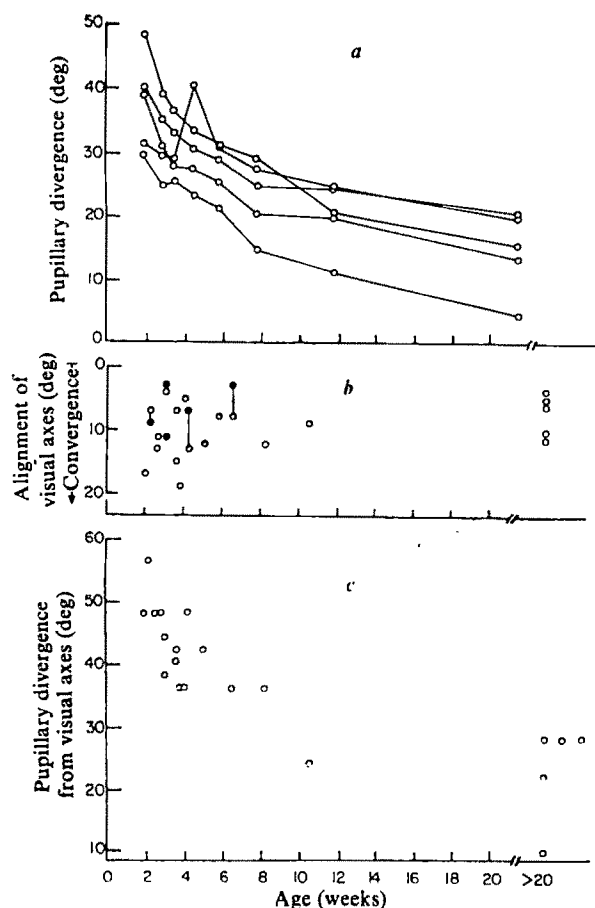
Negatives are processed, mounted in slide holders, and magnified by projection to enable accurate measurements of pupillary divergence. An exact magnification factor is obtained for each photograph by use of a ruler positioned just above the cat's head in the plane of its corneas.

Using the procedure described in step 1, we have measured the angle between the pupillary axes at successive stages of development for a number of kittens. Figure 2a shows these data for five animals. As found previously², there is marked divergence in the youngest kittens which decreases, but does not entirely disappear, with increasing age.

In order to measure visual axis alignment during this period, we have used the four-step procedure described above. Each of 21 animals was studied once at a particular age. By plotting receptive fields (step 4 above), we are able to measure the angle between the visual axes in the paralysed animal, and since we know how interocular alignment has changed between alert and paralysed states (steps 1-3 above), we are able to infer visual axis alignment in the awake cat. Results of this determination are shown in Fig. 2b. Each open circle represents the angle between the visual axes of an alert cat of a given age. The result is markedly different from that which might be anticipated on the basis of measurements of pupillary divergence. From the earliest age, 2 weeks, all cats are convergent rather than divergent. The magnitudes of convergence are somewhat surprising. This may indicate that alert cats converge excessively under our experimental conditions or that a small systematic error is introduced by our method. In either case, two conclusions may be firmly drawn. First, no trend is evident which suggests that visual axis alignment changes with age. Second, for some positions in object space, cats seem able to receive corresponding binocular input at all ages tested.

If visual axis alignment is relatively constant with age but the angle between the pupillary axes changes substantially, then the angle between the visual and pupillary axes must also vary. This angle is given for cats of different ages in Fig. 2c. Each point represents the angle of pupillary axis divergence if visual axes are assumed to be parallel. These data show a close match between the magnitude and time course of the anatomical changes and the differences in pupillary divergence indicated in Fig. 2a.

Before drawing conclusions from these findings, we must consider a few potential difficulties in our methods and the controls devised to deal with them. First, use of the pupil as a reference for mensuration depends on the assumption that its position remains constant throughout our procedures. Constriction may not be symmetrical however, and pupil centres may shift when the necessary local and systemic paralyzing and



ophthalmic drugs are used. Even small changes could cause considerable errors in determining interocular alignment. To provide an alternate fiducial point to the pupil, very small tattoos made with titanium oxide in saline were placed on the corneas of several cats. Measurements of visual axis alignment based on the use of these tattoos are included in Fig. 2b (filled symbols). When both the tattoo and pupil techniques are used for the same cat, the two points are connected by a vertical line (open to filled symbols). It is clear that the data obtained using tattoos are in agreement with those of the other method. Thus, it seems that no systematic errors result from the use of pupils.

Another possible source of error is concerned with the influence of luminance level. To obtain accurate measurements using pupils, it is necessary to cause constriction by use of high luminance or drugs or both. It is possible that the light levels we use may influence our results. In particular, reflex convergence might occur, and this possibility must be thoroughly explored, as we find that cats are somewhat convergent at all ages. Using tattoos, we were able to take photographs with room illumination only as well as under higher luminance levels (from 50 to 3,000 cd m^{-2}), and we determined that no significant light-induced convergence occurred.

Apart from these considerations, our procedures involve other potential sources of error. Cyclotorsional changes could confound measurements of strictly lateral displacements. Eye drift could occur between the time that photographs of the immobilised animal are taken and the time receptive fields are plotted. Contact lenses, which are inserted before receptive field study, could cause displacement of the eye or prismatic changes of the visual axis. We have ruled out each of these possibilities. Cyclotorsional alignment has been measured under all conditions, and our findings are reported elsewhere⁸. Possible effects due to eye drift and contact lens use have been determined

Fig. 2 a, The pupillary axis is defined by a line perpendicular to the cornea that passes through the centre of the eye's entrance pupil (the entrance pupil is the image of the real pupil formed by the cornea). The angle of divergence between the pupillary axes may be obtained from the amount of pupillary divergence measured using photographs. By taking photographs at several angles relative to the cat, we have determined that linear and angular divergences are related by a constant factor (mean for all cats: 15.4 deg mm^{-1}). With the conversion factor and photographs taken before and after immobilisation, it is possible to deduce the angular change in pupillary alignment, and thus in visual axis alignment. Angular divergence of the pupillary axes of the alert kitten decreases with increasing age, as shown for five kittens photographed at regular intervals during development. The distance in mm by which separation of pupil centres exceeds separation of corneal reflexes is converted into degrees through use of the empirically determined conversion factor mentioned above. Each point represents a mean value based on eight photographs. **b,** The angle between the visual axes is operationally determined as follows. The electrode is placed within the Area 17 representation of area centralis. The receptive fields of cells in this region, because they represent homologous retinal points, are separated by the same distance on the plotting screen as those from the centre of the area centralis. Thus the angle between the visual axes is the inverse tangent of the distance between receptive field centres minus inter-pupillary distance divided by the distance between the cat's eyes and the stimulus screen. Visual axis alignment is convergent in cats of all ages. \circ , Represent values based on the four-step procedure illustrated in Fig. 1. \bullet , Depict values obtained by a method in which corneal tattoos were used similarly to the pupils as eye alignment reference points. In cases where pupils and tattoos were used for one animal, \bullet and \circ are joined by a vertical line. **c,** Angular divergence of pupillary axes relative to visual axes (that is pupillary divergence if the visual axes were parallel) decreases with increasing age. Each point is obtained from a single paralysed animal through combined use of receptive field and photographic measurements. Magnitude and time course of the anatomical change illustrated here match those of the change in pupillary divergence shown in (a) above. Data points in (b) and (c) were obtained from the same animals.

to be negligible by photographing several times during paralysis for some cats, monitoring eye positions through ophthalmoscopic projection of the optic discs, and by plotting receptive fields with and without contact lenses in place. Sources of variability inherent in our measurements include those related to photographic processing and the plotting of receptive fields. We have analysed each of these factors and find that the overall variability in our procedures is exceedingly small.

We conclude that the visual axes are not divergent in young, alert kittens. This finding is contrary to what may be inferred from pupillary photography alone, and also from receptive field separation in very young paralysed animals⁹. Because the visual axes of young kittens are convergent, it seems likely that fused binocular vision of objects at some distances occurs during early life. Further work is required to confirm this speculation.

This work was supported by a research grant from the National Eye Institute, U.S. Public Health Service, N.I.H. and a Research Career Development Award from the U.S. Public Health Service to R.D.F.

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Fatty acids, aldehydes and alcohols as attractants for zoospores of *Phytophthora palmivora*

ZOOSPORES of the important plant pathogenic fungi *Pythium* and *Phytophthora* are attracted to plant roots and to exudates and extracts from roots¹. Attraction (positive chemotaxis) to amino acids^{2,3} which occur in root exudates, and ethanol⁴, which will be produced by roots in waterlogged soils, has been demonstrated, but no substance has been found which by itself is as powerful an attractant as root exudate. The attractiveness of root exudates must therefore be explained by additive or synergistic effects between the known attractants, or by powerful attractants which have hitherto escaped detection, or both. There is evidence for additive and synergistic effects⁵, but the fractionation of root exudates has not so far yielded evidence of additional chemotactic factors. We report here the existence of further attractants for fungal zoospores, and consider the possibility that they may be of significance in chemotaxis to plant roots.

Our experiments were carried out with *Phytophthora palmivora* (Butler) Butler, which attacks a range of tropical crops, especially cocoa (*Theobroma cacao* L.), in which it infects pods, roots and other parts⁶. Chemotaxis of zoospores to cocoa root exudates has been demonstrated⁷, as well as to pod extract⁸ and some amino acids^{2,9}. We used, under licence from the Ministry of Agriculture, Fisheries and Food, the same strain that we used in a study on negative geotaxis of zoospores⁸, and a swim-in test in which the number of zoospores entering a capillary tube containing a suspected attractant are counted and compared with numbers entering a control tube¹. Attraction of *Ph. palmivora* zoospores to some amino acids^{2,9} was confirmed, and in addition they were shown to respond, as do *Phytophthora cinnamomi* zoospores¹, to ethanol. Other alcohols, aldehydes and fatty acids were investigated. Allen and Newhook¹ had mentioned that *Ph. cinnamomi* showed chemotaxis to 5 mM solutions of methanol, *n*-propanol, *n*-butanol and acetaldehyde, but of these compounds we find only acetyldehyde active with *Ph. palmivora*. Several alcohols, aldehydes and fatty acids, however, were shown to be attractants, some of them having thresholds considerably lower than those for ethanol or any other attractant so far established for fungal zoospores (Table 1). The most potent attractants had 4–6 carbon atoms, iso- compounds tended to be more effective than those with straight chains, and acids and especially aldehydes were usually more powerful than the corresponding alcohols. After entering the capillary tube, zoospores soon settled, encysted and germinated, although some compounds at high concentrations caused lysis instead of encystment. Isovaleraldehyde, for example, resulted in lysis at 10 mM, some lysis but some encystment and germination at 1 mM, and complete germination in the range 0.001–0.1 mM. We have also found that zoospores of *Ph. palmivora* are repelled by H⁺ and other monovalent ions, as are those of *Ph. cinnamomi*¹². Taking into account these repellent effects, experiments on adjusting the pH of attractants have been carried out. These experiments indicate that the fatty acids are attractive in the unionised (R.COOH) but not the ionised state (R.COO⁻). Hence, the new attractants have the common feature of volatility.

Are these new attractants likely to be of significance in nature? In order to ascertain whether such compounds are important in chemotaxis to plant roots, it would be necessary to fractionate root exudates before chemotactic tests in a way that avoids the loss of volatile components. The procedures so far used are unlikely to avoid such losses and hence volatile attractants might have been overlooked.

Table 1 Results of chemotaxis tests on zoospores of *Phytophthora palmivora*

Carbon atoms	Chemical*	Response†	Threshold (mM)‡
Alcohols			
1	Methanol	0	—
2	Ethanol	+	5
3	Propanol	0	—
3	Isopropanol	+	1
4	Butanol	0	—
4	Isobutanol	+	1
4	Tertiary butanol	+	1
5	Amyl alcohol	+	5
5	Isoamyl alcohol	+	0.1
9	Nonanol	0	—
Aldehydes			
2	Acetaldehyde	+	5
3	Propionaldehyde	+	1
4	Butyraldehyde	+	0.1
4	Isobutyraldehyde	+	0.1
5	Valeraldehyde	+	0.1
5	Isovaleraldehyde	+	0.001
6	Caproaldehyde	0	—
7	Heptanal	0	—
8	Octanal	0	—
9	Nonanal	0	—
Organic acids			
2	Acetic	0	—
3	Propionic	0	—
4	Butyric	?	—
4	Isobutyric	?	—
5	Valeric	+	0.5
5	Isovaleric	+	0.5
6	Caproic	+	0.5
6	Isocaproic	+	0.1
8	Octanoic	0	—

Zoospore suspensions ($1-2 \times 10^5$ spores ml⁻¹) were prepared as previously reported⁸ except that the suspending medium into which spores were released was 0.5 mM potassium phosphate buffer (pH 5.8) + CaCl₂ (0.05 mM) + MgCl₂ (0.05 mM). Capillary tubes (1 µl) were filled with the substance under test dissolved in suspending medium and adjusted if necessary to pH 5.8 with 1 M NaOH, or the suspending medium alone (control) by heat sealing one end of the capillary and allowing the liquid to be drawn into the tube as it cooled¹⁰. A test and a control capillary were placed at opposite ends of an assay chamber (20 × 6 × 1 mm deep) which was then filled with a zoospore suspension and covered with a coverslip. After 10 min, spores were immobilised by gently warming the chamber, and the spores inside the two capillaries were counted. Five replicates were carried out with each concentration of a substance tested and the probability of any higher count obtained with the test capillary as compared with the control capillary being due to chance was determined by means of the *t* test for paired samples¹¹.

*Except where indicated as iso- or tertiary, the straight-chain (*n*) compounds were used.

†Attraction by concentration 10 mM, with $P < 0.01$; 0 = no attraction; ? = apparent attraction towards, but not into, capillary.

‡The lowest concentration of an already proved attractant giving attraction with $P < 0.05$.

Fries¹³ states that with gas chromatography pea roots give 'a rich spectrum indicating the presence of numerous volatile compounds' and cites references establishing the production of volatile alcohols, aldehydes and organic acids (including valeric acid) by plant roots, fruits and other parts. Hence the compounds shown in the present study to be chemotactic may well be present in fresh root exudates and have a role in attraction. This may be true of related compounds, such as unsaturated fatty acids, which are known to be produced by roots¹³ but which we have not tested.

We thank the SRC for a studentship to J.N.C.

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Received 10 October; accepted 16 December 1977.

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Cell division factors (cytokinins) from irradiated plant tissue

REPORTS on the effects of ionising radiation on plant cells in callus culture are quite limited. Work on a few species¹⁻³ has shown that low doses of γ radiation, starting at 0.5 krad, stimulate callus growth and differentiation. Larger doses ranging from a few krad to about 20 krad inhibit growth and differentiation, respectively. High doses (16 krad and above) have been shown to alter culture media components chemically and thereby affect cell growth⁴ and differentiation⁵. We now report that

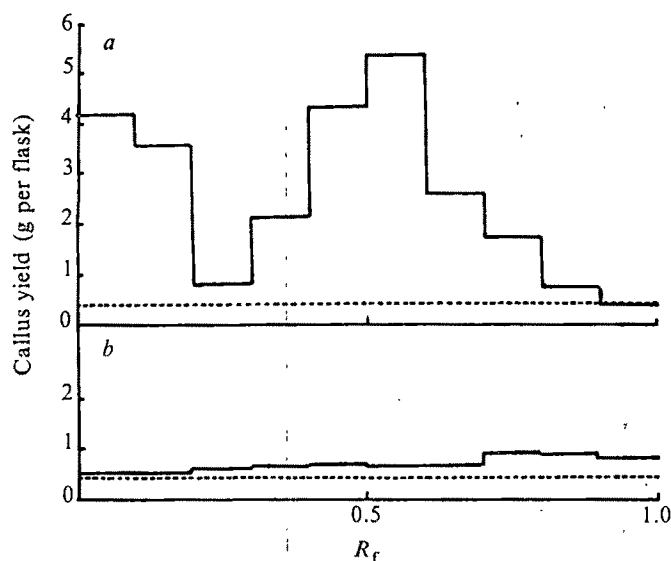


Fig. 1 Tobacco callus cytokinin bioassay of paper chromatographic R_f zones from extracts (140 g equiv. fresh weight) of irradiated (1-2 krad) (a) and nonirradiated (b) *Haworthia* tissue. Tissue (0.4 kg) was blended in a Waring blender with sufficient cold absolute ethanol to obtain a final ethanol concentration of 70% assuming all the tissue to be water. The mixture was shaken in the cold for 8 h and filtered through gauze. The solid residue was resuspended in additional cold 70% ethanol, shaken for 4 h and filtered. Filtrates were combined, adjusted to pH 2.5 with HCl and passed through a Dowex 50W-X8 cation exchange column (2.9 \times 15 cm, 50-100 mesh, H⁺ form). The column was washed with 150 ml of distilled water and cytokinins were eluted with 150 ml of 2M NH_4OH followed by 350 ml of 6M NH_4OH . Ammonia was removed from the eluate under an air stream and the remaining aqueous solution was taken to dryness in a vacuum. The dry residue was extracted with 95% ethanol and the ethanolic solution was reduced to a small volume and strip loaded on Whatman 3MM paper. Ascending chromatography was carried out overnight using 2-butanol: concentrated (28% NH_3) NH_4OH (4:1, v/v) and chromatograms were dried, cut transversely into 10 equal R_f strips and eluted with 95% ethanol. Aliquots from each strip were bioassayed for cytokinin activity. Samples for bioassay were dried in a vacuum in 50-ml flasks; 25 ml of nutrient agar medium^{8,9} was added to each, and the flasks were autoclaved (20 min at 18 pounds per inch²). Three pieces of Wisconsin 38 tobacco pith callus (approximately 150 mg per piece) were placed in each flask and the cultures were maintained in diffuse light at $23^\circ \pm 2^\circ \text{C}$. After 5 weeks, tissues were collected and fresh weights were recorded. The broken lines show callus yield on basal bioassay medium; kinetin at $5 \mu\text{g l}^{-1}$ yielded 3.01 g of tobacco callus.

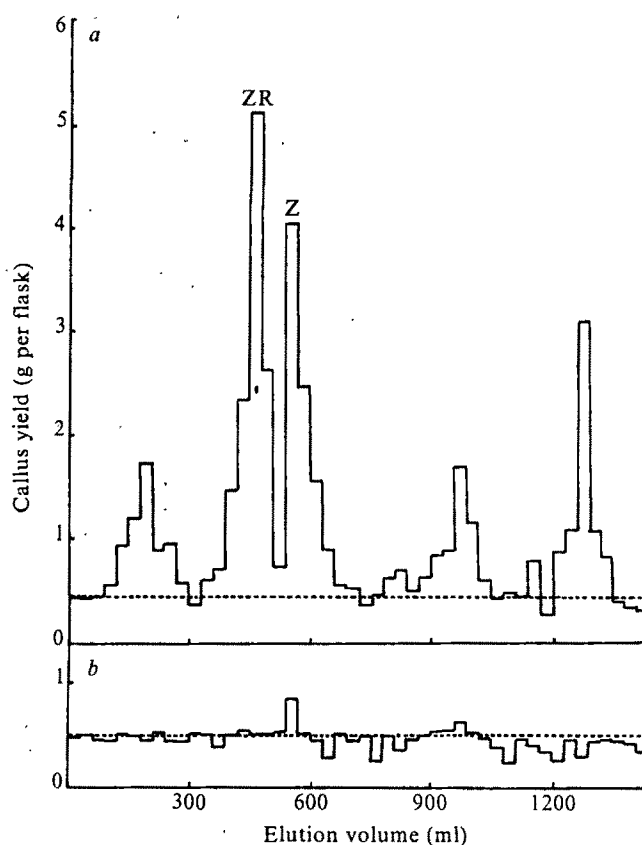


Fig. 2 Tobacco callus cytokinin bioassay of Sephadex LH-20 column fractions (25 g equiv. fresh weight) after chromatography of paper R_f zones 0.4-0.8 (Fig. 1) from irradiated (a) and nonirradiated (b) *Haworthia* tissue. Eluates of paper R_f zones 0.4-0.8 from irradiated tissue were taken to dryness and the residue was dissolved in 1 ml of 35% ethanol and loaded onto a Sephadex LH-20 column (2.5 \times 90 cm). The column was eluted with 35% ethanol and forty-eight 30-ml fractions were collected and bioassayed for cytokinin activity according to the procedure described in Fig. 1. Authentic samples of zeatin and zeatin riboside were chromatographed in the same conditions and their elution positions are indicated by Z and ZR, respectively. The broken lines show callus yield on basal bioassay medium; $5 \mu\text{g l}^{-1}$ kinetin yielded 3.42 g of tobacco callus.

gamma irradiation (1-2 krad) of *Haworthia* in callus culture results in the formation of cytokinins⁶—cell division factors—not detected in nonirradiated tissue.

Tissue was excised from surface sterilised inflorescence stem segments of *Haworthia mirabilis* Haw. and cultured on Murashige-Skoog medium⁸ supplemented with synthetic cytokinin (kinetin, 6-furfurylaminopurine, 0.2 mg l^{-1}) and auxin (naphthaleneacetic acid, 0.2 mg l^{-1}). Callus which developed was maintained by subsequent subculture on the same medium containing kinetin and naphthaleneacetic acid (both 1.5 mg l^{-1}). All tissue was kept at $23^\circ \pm 2^\circ \text{C}$ under continuous light.

Callus tissues weighing approximately 350 mg were irradiated using a ^{60}Co γ source. Tissue given 1-2 krad at a dose rate of 0.19 krad min^{-1} grew continuously, became compact and did not differentiate. It could be subcultured repeatedly on hormone (cytokinin and auxin) and *myo*-inositol-free media and showed no diminution of growth. Nonirradiated callus required hormones or *myo*-inositol for continued growth.

Cytokinins were isolated from irradiated tissue 8-10 weeks after third generation subculture on hormone and *myo*-inositol-free medium by a procedure similar to that described for *Populus \times robusta* leaves⁷. Cytokinins were assayed as described in the legend to Fig. 1. Nonirradiated tissue cultured for 8-10 weeks on medium containing kinetin and naphthaleneacetic acid (both 1.5 mg l^{-1}) was analysed for cytokinins in the same way. Figure 1 shows results of cytokinin bioassay of extracts of irradiated and nonirradiated tissue after paper chromatography. There were two zones of activity from irradiated tissue, one at

R_f 0.1–0.2 and a larger response from R_f 0.4 to 0.8. No corresponding response was obtained from nonirradiated tissue. R_f zones 0.4–0.8 from both irradiated and nonirradiated tissue were further purified on a Sephadex LH-20 column using 35% ethanol¹⁰, as described in the legend to Fig. 2.

Five areas of cytokinin activity were obtained from irradiated tissue after chromatography on Sephadex LH-20 (Fig. 2a). The two major peaks centred in fraction 16 and 19 cochromatographed with authentic samples of zeatin riboside (6-(4-hydroxy-3-methyl-2-butenylamino)-9- β -D-ribofuranosylpurine) and zeatin, respectively. Authentic samples of 6-(3-methyl-2-butenylamino) purine and its riboside, both known cytokinins, did not cochromatograph with any peaks of cell division activity from irradiated tissue. Essentially no cytokinin activity was obtained from nonirradiated tissue except a small response in fraction 19 which cochromatographed with zeatin (Fig. 2b). Bulk fractions (200–300 ml each) beyond those shown in Fig. 2 were collected until a total effluent volume of approximately 2,200 ml had passed through the column in the case of the irradiated and of the nonirradiated tissue. Bioassay of these fractions showed no additional cytokinin activity eluting from the column. Chromatography of R_f 0.1 and 0.2 from irradiated tissue on Sephadex LH-20 using the same conditions as described in Fig. 2 yielded a single peak of cytokinin activity centred on fraction 14. This compound eluted before authentic zeatin riboside, our earliest eluting standard cytokinin.

In conclusion, our findings demonstrate that gamma irradiation of plant tissue (*Haworthia*) in callus culture results in the formation of cell division factors or cytokinins. The origin of these compounds and the reason for the diversity of cytokinins in irradiated tissue remain to be clarified. It has been proposed¹¹ that free cytokinins are produced by a pathway that includes synthesis and degradation of cytokinin-containing transfer RNA. The cytokinin content of transfer RNA from irradiated and from nonirradiated tissue is being investigated.

We thank Ms J. Richardson for technical assistance.

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Received 31 October; accepted 5 November 1977.

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Chromosomal rearrangement involved in insecticide resistance of *Myzus persicae*

POPULATIONS of the peach-potato aphid *Myzus persicae* (Sulzer) heterozygous for a particular translocation between autosomes 1 and 3 were first discovered in glasshouses in Great Britain¹. A similar or identical translocation was subsequently found to occur commonly in field populations of *M. persicae* in many parts of the world (Table 1). In tropical and warm temperate regions *M. persicae* is permanently thelytokous², but survival of translocation heterozygotes in the sexual cycle of the aphid,

where a translocation would be expected to impose a severe genetic load³, has also been demonstrated⁴. Considerable resistance to organophosphorus insecticides is common in glasshouse populations of *M. persicae*⁵, and laboratory clones originating from glasshouses and isolated for such resistance are invariably translocated. A link between the translocation and organophosphorus resistance has been suspected, as this could provide the selective advantage which would explain the worldwide occurrence of translocation heterozygotes and their survival in the breeding system. However, no information has been available on the comparative resistance of translocated and normal aphids from most parts of the world. Populations with moderate organophosphorus resistance that appeared in the field on sugar beet in England in 1974 (ref. 6) had the normal karyotype, as also did a resistant clone originating from peaches in France in 1968. Now we report evidence that directly implicates the translocation in the organophosphorus resistance of *M. persicae*.

In Japan, 17 clones of *M. persicae* were tested for their resistance to malathion using a dry film method⁷, with 10 apterae per vial (4×2 cm diameter). Mortalities were recorded 24 h after treatment. The six clones of normal karyotype showed greatest susceptibility to malathion (Table 2). Eight clones with the A1,3 translocation, including a laboratory-reared triploid⁴, showed various degrees of resistance, and one of these had a resistance factor of more than 100. The remaining three clones were heterozygous for an autosome 3 dissociation, which could be the first step in the rearrangement⁴. These also showed different degrees of resistance to malathion.

In England, 100 F₁ clones were obtained from a cross between sexual females (oviparae) from a karyotypically normal, organophosphorus-susceptible clone ('U'), and males from a translocated, very resistant clone (GCRI-1). The latter clone, which was approximately 100 times as resistant to dimethoate as the susceptible parent (U), was derived from a population on chrysanthemums in a glasshouse at Littlehampton, Sussex, and is believed to be a widely distributed clone of *M. persicae* in glasshouses in southern England. Eggs were overwintered in a well-ventilated cage in insectary conditions, and the hatching fundatrices were matured on radish plants and transferred to excised potato leaves in individual cages when adult, to give rise to separate F₁ clones.

Table 1 Occurrence of A1,3 translocation heterozygotes in samples of *M. persicae* collected 1972–1977

Origin of samples and years of collection	No. of samples examined	No. of samples with translocation heterozygotes
Great Britain		
glasshouses (1974–77)	15	15
field (1972–76)	48	1
Rest of Europe		
glasshouses (1972–76)	5	4
field (1972–76)	6	0
Rest of World (field only)		
Japan (1974–76)	31	18
Hong Kong (1976)	1	1
Philippines (1975)	2	2
Australia (1975–77)	18	7
New Zealand (1975)	3	1
Kenya (1975)	7	5
Nigeria (1975)	1	0
Rhodesia (1975)	1	0
South Africa (1977)	1	1
Canada (1975)	2	1
California (1975–77)	6	5
Michigan (1975)	17	16
Ohio (1975)	2	1
Brazil (1975)	1	1
Chile (1976–77)	2	0

Table 2 Resistance to malathion in relation to karyotype of 17 clones of *M. persicae* in Japan

Clone	Origin	Karyotype	LD ₅₀ per (μ g per vial)	Fiducial limit ($P = 0.05$)	Resistance factor
URY-0	Field, on <i>Raphanus</i>	Normal	0.58	0.56-0.61	1.00
KM602	Field, on <i>Prunus</i>	Normal	0.69	0.55-0.85	1.18
UM604	Field, on <i>Prunus</i>	Normal	0.70	0.65-0.75	1.20
14-1	Laboratory reared	Normal	0.71	0.65-0.78	1.22
25-1	Laboratory reared	Normal	0.85	0.82-0.90	1.46
622-2	Laboratory reared	Normal	1.19	1.09-1.30	2.05
JCG-1	Field, on <i>Brassica</i>	A3 dissociation	1.72	1.55-1.92	2.95
UMG7501	Field, on <i>Prunus</i>	A1,3 translocation	1.93	1.63-2.29	3.31
622-1	Laboratory reared	A1,3 translocation	2.00	1.81-2.20	3.43
SCG-11	Field, on <i>Brassica</i>	A3 dissociation	2.43	2.41-2.47	4.16
HHR-1	Field, on <i>Brassica</i>	A1,3 translocation	2.89	2.16-3.94	4.96
622-5	Laboratory reared	A1,3 translocation	3.11	2.85-3.41	5.34
521-11	Laboratory reared	Triploid, with A1, 3 translocation	3.84	3.10-4.76	6.59
SCG-1	Field, on <i>Brassica</i>	A1,3 translocation	5.67	4.45-7.23	9.73
521-9	Laboratory reared	A3 dissociation	9.24	6.59-13.02	15.85
JCR-1	Field, on <i>Brassica</i>	A1,3 translocation	9.24	6.58-12.92	15.86
URR-0	Field, on <i>Raphanus</i>	A1,3 translocation	60.19	47.02-77.04	103.24

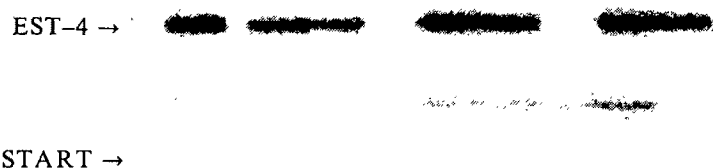
Thirty-five of the 100 F_1 clones were heterozygous for the A1,3 translocation. The other 65 were all of normal karyotype. Deviation from an expected 50:50 ratio⁴ was perhaps due to a hatching delay in aphids carrying the translocation; the clones were set up in approximately chronological order as the fundatrices matured, and only nine of nos 1-50 were translocated, compared with 26 out of nos 51-100.

Starch gel electrophoretograms were prepared from whole-body homogenates of four or five individual adult virginoparae (total weight 1.9-2.1 mg) from each clone, and stained for esterases⁸. All clones were examined in either the third or fourth generation after the fundatrix. There is good genetic⁹ and biochemical¹⁰ evidence that organophosphorus resistance in *M. persicae* is linked with increased carboxylesterase (est-4) activity. All 65 F_1 clones which were karyotyped as normal showed negligible est-4 activity, as in the normal, susceptible parent clone U (Fig. 1). Twenty-eight of the 35 clones which had the A1,3 translocation showed increased est-4 activity, although some samples clearly had less est-4 than the translocated, resistant parent clone, GCRI-1. The remaining seven translocated F_1 clones had est-4 activity characteristic of susceptible aphids. When additional samples of these seven clones were tested, using homogenates of single aphids, it was found that there were some individuals in each clone with increased est-4 activity (Fig. 2). Further studies revealed that est-4 activity varied between individuals in several other translocated F_1 clones, although there were some, like the parent clone GCRI-1, with a stable, high level of est-4.

Thus the translocation and the resistance mechanism show complete linkage, at least when inheritance is through the male. Spermatogenesis may normally be achiasmate in *M. persicae*, as seems to be the case in two species of *Eucera*¹¹. Alternatively, the translocation may maintain its association with resistance by inhibiting crossing over. The question remains as to whether crossover suppression alone is sufficient to explain the frequency of the translocation in natural populations of *M. persicae*. It seems more probable that the translocation results in an advantageous rearrangement of genes contributing to the resistance mechanism. Preliminary studies of inheritance of organophosphorus resistance in *M. persicae* clones without the translocation indicate that at least two alleles are concerned in the regulation of est-4 activity⁹, although it is not known how many loci are involved. The translocation may bring resistance-associated genes which were previously on autosomes 1 and 3 into the same linkage group.

Linkage would be an advantage in itself, but there is some evidence that the chromosomal rearrangement actually enhances resistance by directly affecting gene action or interaction. First, *M. persicae* clones with the greatest organophosphorus resistance and est-4 activity, when examined cytologically, have invariably been translocated. Clone GCRI-1, with greater than 100-fold resistance, is nevertheless only heterozygous for genes conferring high est-4 activity. Second, the resistance of Japanese clones of *M. persicae* with the autosome 3 dissociation could be due to the inactivation of a regulatory locus on this chromosome. Third, the type of intraclonal variation illustrated by Fig. 2 is suggestive of a variegated (V-type) position effect¹²,

Fig. 1 Electrophoretogram showing linkage between a chromosomal translocation and increased est-4 activity in *M. persicae*. Parent clones GCRI-1 and U are on the extreme left and right of the gel respectively. In between are 16 representative F_1 clones, the eight to the left being translocated and the eight to the right being of normal karyotype. Each sample is the homogenate of four or five aphids. Starch gel, stained with Fast Blue BB salt using 1-naphthyl acetate as substrate.



EST-4 →

START →

Fig. 2 Electrophoretogram showing variability of gene expression at the *est-4* locus between 16 individuals in one F₁ clone (TN 74) of *M. persicae*. Each sample is the homogenate of a single adult aphid. Individuals of the parent clone GCRJ-1 are placed at both ends of the gel for comparison. Starch gel, stained with Fast Blue BB salt using l-naphthyl acetate as substrate.

such as might occur if resistance-associated loci were close to the point of translocation, and thus liable to inactivation by repositioned heterochromatin. V-type position effects classically explain variations in gene expression between cells within an individual, but could apply equally to differences between individuals within a clone. The extent of variegation in any one clone would be subject to the influences of modifiers in the genetic background, so that in different F₁ clones the expression of genes subject to position effect could vary either within individuals or between individuals in a single generation, or changes could occur over many generations. Intracolonial variation due to V-type effects would clearly complicate the results of insecticide bioassays, and might explain the widely different LD₅₀ values obtained from translocated clones in the resistance tests in Japan (Table 2). It could also explain the instability of organophosphorus resistance within certain clones of *M. persicae*^{8,13-15}.

Further work is necessary to substantiate the position effect hypothesis. The variable activity of the *est-4* locus in translocated clones of *M. persicae* could prove a useful tool for studying certain aspects of gene expression.

We thank J. Martin and A. Sutton for technical assistance, and Drs M. Ashburner and G. Hewitt for commenting on the manuscript. We regret that there is insufficient space to thank individually all those who sent *M. persicae* to England for karyotyping.

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Received 22 August; accepted 5 December 1977.

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Does carcinogenic potency correlate with mutagenic potency in the Ames assay?

AN approximately linear relationship between the mutagenic potency of a chemical in the *Salmonella* reverse mutation assay of Ames¹ and its carcinogenic potency in animals, was recently alluded to² and has since been discussed at several international symposia^{3,4}. The implications of such a claim are clearly momentous. At present, the fact that the *Salmonella* assay can give both false positive and false negative results when compared with animal tests^{6,7} has led to its being regarded as an early warning system rather than the final arbiter of animal and possibly human carcinogenicity. A correlation between mutagenic and carcinogenic potency would, however, mean that a strong positive response in the Ames assay would define a potentially potent carcinogen, and would suggest that some compounds give positive results in this assay but fail to induce cancer in animals because their mutagenic potency is too low. We believe, however, that there is sufficient experimental evidence to treat any superficial potency correlation with great caution at this stage.

Our reservations about this correlation are based on the following. The concept that it is the absorption, distribution, metabolic activation and resultant half life of derived active species, rather than the *in vitro* mutagenic potency of a compound which determines its carcinogenic potency has been discussed by others^{8,9}. Also, some compounds are known to display species-dependent biological (including carcinogenic) properties¹⁰, yet it is from such species-dependent carcinogenicity studies that a potency correlation would have to be derived. Thus, a compound may be potentially carcinogenic as defined by an *in vitro* test yet be non-carcinogenic in the particular strain of species of animal in which it has been evaluated.

Apart from the above concerns in *in vivo* animal studies, serious doubts can be raised about the implied absolute quantitative significance of a *Salmonella* assay revertant colony count. In most cases, compounds which are mutagenic and/or carcinogenic require metabolic activation before they can elicit their effect. The *in vitro* medium usually employed for this activation is a sub-fraction of an homogenate of induced rat liver, the S9 microsomal mix. Despite its efficiency in a general sense (activation against no activation), the results mediated by this preparation are not generally regarded as being quantitatively significant. Also, although the general method of preparation and use of the S9 mix has been described¹, individual variations in protocol detail are common¹¹. If the assay results mediated by an enzyme preparation are to be quantitatively assessed then the enzyme preparation itself should first be calibrated in terms of the presence and activity of at least some of the basic activation and deactivation enzymes.

This is rarely, if ever, undertaken. Similarly, liver enzyme inducing agents such as Aroclor 1254, phenobarbitone or methylcholanthrene are used specifically to increase the levels or activity of certain enzymes, but in general no attempt is made to either quantitate this increase or to assess if it invariably leads to an increased mutagenic response.

The quantitative problems posed by unquantified and uncalibrated enzyme preparations can be illustrated by several recent studies. The carcinogen benz(a)pyrene (Fig. 1, I) requires metabolic transformation before it can elicit a carcinogenic or mutagenic effect. Although initial studies implicated the 4,5-epoxide (Fig. 1, II) (the K-region epoxide) as the critical intermediate^{12,13}, recent studies indicate that it is probably the 7,8-diol-9,10-epoxide derivative (Fig. 1, III) which is the important chemical species in cancer induction^{12,14-16}. The 7,8-diol group itself probably arises by attack on an intermediate 7,8-epoxide by epoxide hydratase enzymes¹⁴. It is, therefore, clear that the S9 mix used to activate benz(a)pyrene in an *in vitro* assay will be required to possess both oxidative enzymes for epoxide formation (such as cytochrome P-450) and epoxide-destroying enzymes, such as epoxide hydratase. In addition, various glutathione-S-transferase enzymes will be present in the S-9 mix and may react with any epoxide groupings present. It should therefore be possible to manipulate the muta-

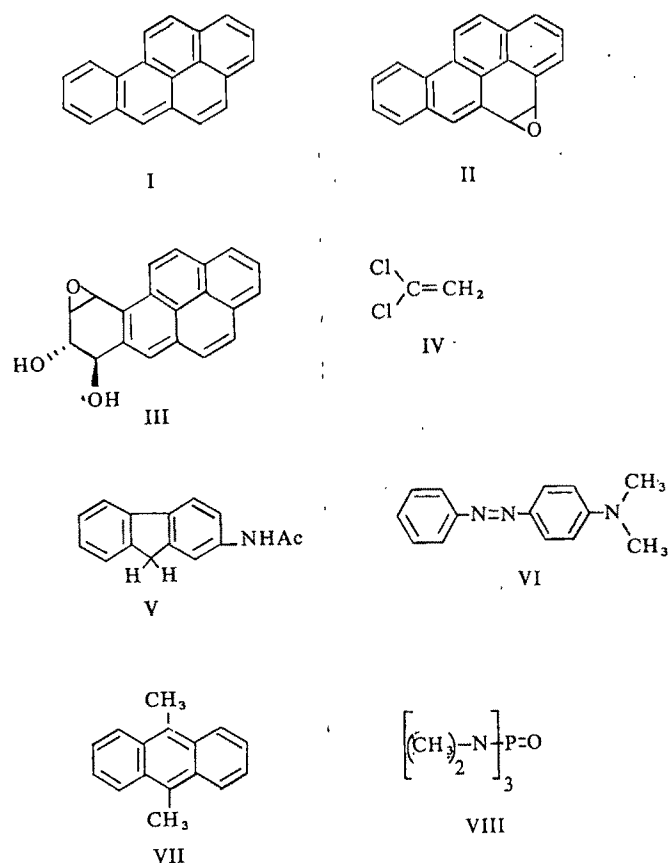


Fig. 1 Structures of test compounds.

genic response obtained for benz(a)pyrene in an *in vitro* test simply by varying the relative levels or activities of these three enzymes, and this is observed. Figure 2 shows the results obtained by Oesch *et al.* for benz(a)pyrene in the Ames assay^{17,18}. As expected, the addition of pure epoxide hydratase enzyme essentially abolished the mutagenic response while the chemical inhibition of this enzyme greatly enhanced the observed mutagenic response. Two factors are important here; first, both of the relevant enzymes are present but generally unquantified in the rat liver S9 preparations used for routine *in vitro* testing. Unsuspected variations in their levels or activities could dramatically alter the mutagenic potency observed for a compound competitively using these enzymes. Second, compound III is a poor substrate

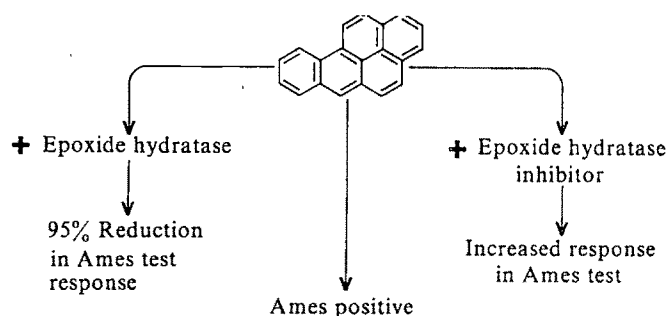
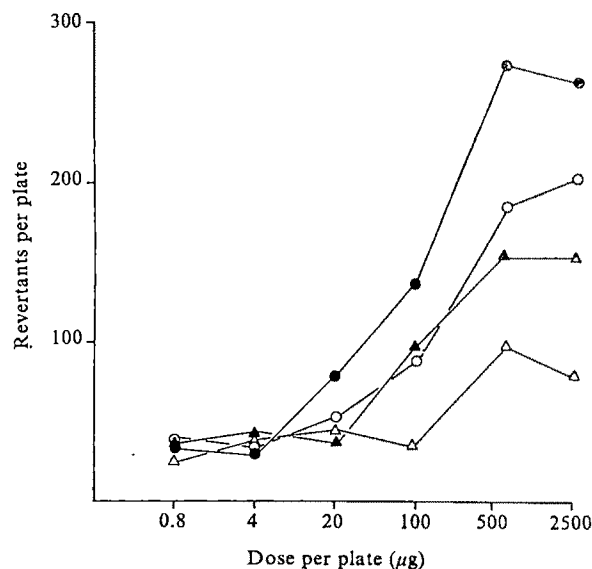


Fig. 2 Benz(a)pyrene action in Ames assay. Adapted from Glatt *et al.*¹⁷ and Oesch *et al.*¹⁸.

for epoxide hydratase¹⁴, which means that the mutagenic potency variations observed by Oesch were probably arbitrarily associated with the K-region epoxide¹⁹. A study of a series of 37 polycyclic aromatic hydrocarbon carcinogens²⁰ also revealed the overall lack of a correlation between carcinogenic potency and mutagenic potency in the Ames assay for that particular series of compounds. Similar findings have also been obtained for a series of structurally related nitrosamines²¹, while Bartsch has reported that vinylidene dichloride (Fig. 1, IV) is mutagenic in the Ames assay when using mouse derived S9 but almost inactive when using rat derived S9²². In the latter situation it is important to consider which result would be the relevant one to quote quantitatively *vis-à-vis* any possible animal carcinogenicity data. Further, the possibility that rats may possess the ability to detoxify epoxides more efficiently than do mice may account for the fact that trichloroethylene produces tumours in mice but not in rats²⁰ (the fact that the carcinogenicity of trichloroethylene has been suggested to arise from the butene oxide stabiliser present in the material tested²⁴ does not alter this argument).

This raises the question as to whether potency correlations should be applied within a given species of animal (for example, comparing the mutagenic potency of a compound in the Ames assay using hamster derived S9, with carcinogenicity data also derived in hamsters). The dangers inherent in this practice have been vividly demonstrated by McGregor, who showed²⁵ that cotton rat derived S9 was the best of eight different liver preparations, variously derived from five different species of animal, for the mutagenic activation of acetylaminofluorene (Fig. 1, V) in the Ames assay. Nonetheless, the cotton rat is

Fig. 3 Mutagenic response of *S. typhimurium* TA 1538 to benz(a)pyrene using Aroclor 1254 induced (○) and uninduced (●) mouse liver S9 mix and Aroclor 1254 induced (△) and uninduced (▲) rat liver S9 mix. Bacteria were obtained from B. N. Ames. Induction and preparation of S9 mix according to Ames *et al.*¹. The experimental protocol followed in all of these experiments is the same as in our earlier studies⁷.



claimed to be resistant to acetylaminofluorene-induced cancer²⁵. A further consideration is the diet employed during a carcinogenicity study or in animals from which livers are to be obtained for an S9 homogenate. The induction of liver tumours in rats with butter yellow (Fig. 1, VI) is dependent on the levels of the deactivating azoreductase enzymes in their livers. By reducing riboflavin intake in the diet, the levels of the riboflavin-dependent azoreductase enzymes are lowered, with a corresponding increase in tumour yield (that is, an apparent increase in carcinogenic potency)²⁶. It would therefore be expected that in the S9 mix used to evaluate related azo-compounds in the Ames assay, the levels of these enzymes would be both variable (by design or by chance) and critical to the apparent mutagenic potency observed. Similar nutritionally induced variations in liver cytochrome P-450 levels, with a resultant variation in enzyme-mediated compound metabolism, have also been reported¹⁰.

In an attempt to demonstrate clearly the dangers of detailed extrapolation of data generated in an *in vitro* test to the possible *in vivo* situation, we conducted a series of experiments with benz(a)pyrene. Five separate S9 fractions were prepared from uninduced and induced rat liver (Sprague-Dawley, Alderley Park strain), induced and uninduced mouse liver (Alderley Park strain, albino) and uninduced guinea pig. The results obtained in the Ames assay using these S9 fractions are shown in Figs 3 and 4. The guinea pig S9 fraction is incapable of effectively activating benz(a)pyrene into a mutagen while both the rat and mouse liver S9 fractions are variously effective. Thus, in this case of benz(a)pyrene at least, induction of the mouse and rat liver enzymes resulted in a reduction rather than an increase in the observed mutagenic potency.

In the supernatant of the S9 fractions there are glutathione-S-transferase enzymes and other chemical species which could react with epoxide intermediates and thereby inactivate them as mutagens. To investigate this possibility we removed the supernatant material from the guinea pig S9 fraction, leaving a pure microsomal fraction (105,000g fraction), and retested benz(a)pyrene using these microsomes in place of the original S9 fraction. The resultant positive effect is shown in Fig. 5. It can be inferred that the supernatant material of the guinea pig S9 fraction contained epoxide-destroying species which had been masking the mutagenic effect of any microsomally derived epoxides. Finally, the same guinea pig supernatant material was added to the microsomes (105,000g fraction) derived from the initial mouse S9 fraction and the reconstituted mouse/

Fig. 4 Mutagenic response of *S. typhimurium* TA 1538 to benz(a)pyrene using S9 mix made from uninduced mouse liver (Δ) and uninduced guinea pig liver (\bullet).

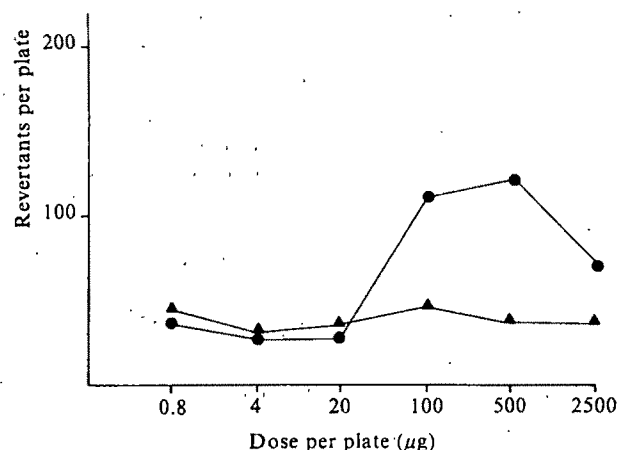
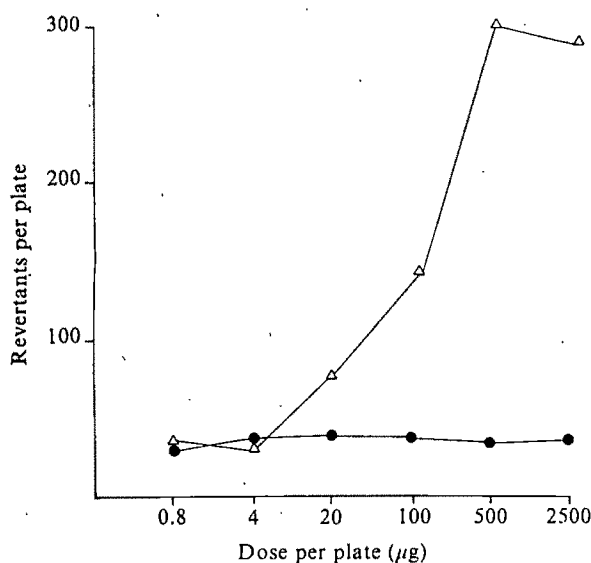


Fig. 5 Mutagenic response of *S. typhimurium* TA 1538 to benz(a)pyrene using guinea pig liver microsomes (105,000g fraction) with added cofactors (\bullet) and uninduced mouse liver microsomes (105,000g fraction) with supernatant from guinea pig (30,000g fraction) and co-factors (Δ).

guinea pig S9 fraction used to re-evaluate benz(a)pyrene. As expected, a negative response was obtained (Fig. 5). Thus, by simple fractionation of the S9 mix, the initial guinea pig negative response and the initial mouse positive response for benz(a)pyrene were both capable of reversal. These results, which are admittedly somewhat contrived in terms of the S9 preparations used for their generation, cannot be discounted as irrelevant because any metabolic activation system is presently regarded as acceptable as long as it can produce a positive result for a chemical.

The above experiments demonstrate that both mouse and guinea pig microsomes are potentially able to activate benz(a)pyrene into a mutagen, but that in a routine assay the observed result is strongly influenced by the surroundings in which the activation occurs, in this case the supernatant fluid. In the context of such sensitivity it should be anticipated that significant variation will occur in any biological parameter, such as mutation or cancer induction, when *in vivo* and *in vitro* responses are compared. A new dimension has recently been added to this debate by the co-mutagenicity effects described by Sugimura *et al.*²⁷⁻²⁹. By testing carcinogens such as butter yellow (Fig. 1, VI) in the presence of the non-mutagens harman or norharman, the mutagenic potency observed for the carcinogen can be dramatically increased (up to 40-fold in one case)²⁸. Further, compounds such as aniline and *ortho*-toluidine showed mutagenic properties under these conditions while being negative in the normal Ames assay²⁹. Whatever the explanation for these observations, their effect on an apparent potency correlation could be critical.

The variations in observed mutagenic potency of a chemical in the Ames assay discussed above are in general limited within a 100-fold range, which may seem negligible to an assay which has a 10⁶-fold mutagenic potency range². In our laboratory, however, this assay only has a 10³-fold range of mutagenic potency⁷. The above mentioned variations are, therefore, highly significant. There are also a sufficient number of exceptions to a potency correlation to discourage its rapid acceptance. For example, the very weak carcinogen 9,10-dimethylanthracene (Fig. 1, VII) (ref. 30) is a potent mutagen in the Ames assay⁷. In contrast, the potent rat carcinogen hexamethylphosphoramide (Fig. 1, VIII) (ref. 31) is usually negative in this assay³², but is mutagenic as evidenced by a positive response given by this compound in *Drosophila*³³. The evaluation of potency correlations may be important when attempting to understand the mode of action and tissue or species specificity of individual carcinogens and mutagens^{34,35}. The premature acceptance of

an invariable correlation, however, may well reduce or at least impede the ultimate acceptance and beneficial impact of the Ames and related assays.

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Received 23 September; accepted 30 November 1977.

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Metabolic deactivation of mutagens in the *Salmonella*-microsome test

THE *Salmonella typhimurium*/mammalian microsome test of Ames *et al.*¹ has provided a simple and sensitive short term assay for the detection of environmental mutagens. Moreover, there is excellent correlation between a positive reaction of the compounds so far tested in this prokaryotic test system and their carcinogenicity²⁻⁴. Metabolic activation of precarcinogens is achieved by incubating, on Petri dishes, the compounds to be tested, the bacterial strains and liver homogenates together with a NADPH generating system¹. The opposite phenomenon, that is conversion from mutagenically active to inert compounds, is also widely known to occur *in vivo*^{5,6} and inactivating enzymes have also been identified for some reactive metabolites⁷. However, the possibility of checking deactivation of the mutagenic potential of chemicals by means of the same *in vitro* system set up for metabolic activation has received little attention. The results reported here provide evidence that some compounds which are mutagenic *per se* for *S. typhimurium* can be partially or totally deactivated in the presence of liver homogenates. This possibility should be considered when results of the *Salmonella*/microsome assay are interpreted, and correlated with animal and epidemiological data.

The compounds tested were sodium azide, sodium dichromate and sodium nitrite (Merck), 5-nitro-2-furoic acid (Ega-Chemie), and captan (*N*-trichloromethylthio-tetrahydro-phtalimid) (Serva). 2-Aminofluorene (Ega-Chemie) was tested to check the activity of the rat microsomal preparation used. Solutions and

Table 1 Mutagenicity of some compounds for *S. typhimurium* with and without S-9 mix

Compound	Amount per plate (µg)	No. of TA100 revertants	
		S-9—	S-9+
Controls	0	226.3 ± 19.5*	204.6 ± 16.3*
2-Aminofluorene	1.25	237	585
	2.5	215	840
	5	242	1,075
	10	229	1,320
Sodium dichromate	10	312	198
	20	530	209
	40	825	231
	80	1,230†	980
Sodium azide	0.625	324	226
	1.25	710	284
	2.5	1,240	530
	5	2,270	1,085
Sodium nitrite	2,500	439	219
	5,000	585	373
	10,000	770	518
Captan	1.25	325	223
	2.5	397	284
	5	570	421
	10	1,115	645
5-Nitro-2-furoic acid	12.5	332	218
	25	845	307
	50	1,380	615
	100	384†	990

*Mean ± s.d.

†Toxic effects (small colonies and spare background lawn).

serial dilutions of compounds were made in water (sodium azide, dichromate and nitrite, and 5-nitro-2-furoic acid) or in dimethylsulphoxide (captan and 2-aminofluorene) and assayed by the plate incorporation test described by Ames *et al.*⁸. TA100 was used as bacterial tester strain. All assays were carried out in the absence and in the presence of the liver microsomal fraction (S-9 fraction) prepared from rats induced with a polychlorinated biphenyl mixture (Aroclor 1254, Monsanto), mixed in various amounts (10-50 µl per plate) with a fixed amount (0.5 ml per plate) of a NADPH-generating system (S-9 mix).

As shown in Table 1, 2-aminofluorene was mutagenic for strain TA100 of *S. typhimurium*, but only after addition of the liver microsomal preparation, while the other compounds were mutagenic *per se*, with dose-response effects. However, addition of the S-9 mix, containing 50 µl of S-9 fraction, resulted in a significant decrease of revertant colonies. The loss of mutagenic response was complete when test compounds were given at low doses, while larger amounts exceeded the deactivating capacity of the S-9 mixture used, with the exception of sodium

Table 2 Relationship between the amount of S-9 fraction embedded in the soft agar overlay and the number of TA100 revertants induced by mutagenic compounds

Compound	Amount per plate (µg)	Amount of S-9 fraction per plate (µl)						
		0	10	20	30	40	50	
Controls	0	234	241	215	223	218	209	
2-Aminofluorene	20	216	325	810	1,430	1,725	2,095	
Sodium dichromate	40	705	420	370	283	228	221	
Sodium azide	1	645	397	302	251	234	237	
Sodium nitrite	5,000	568	454	396	361	358	341	
Captan	2	845	570	436	390	327	296	
5-Nitro-2-furoic acid	20	1,035	640	529	451	385	337	

dichromate, which was totally deactivated below toxic concentrations. The metabolic deactivation of the compounds tested, as well as the metabolic activation of 2-aminofluorene, was correlated with the amounts of liver microsomal fraction embedded in the soft agar overlay (Table 2). Conversely, the number of revertants was not significantly affected by addition of S-9 cofactors without microsomal fraction.

Of the compounds studied, sodium azide, sodium nitrite, captan and 5-nitro-2-furoic acid had been included in the group of 'false' positive mutagens^{2,3}, that is of chemicals which were found to be mutagenic for *S. typhimurium* and gave negative or ambiguous results in animal tests. The reduction of the mutagenic activity in the *Salmonella* test system, in the presence of liver microsomal fractions, might account for the discrepancies between these *in vitro* and *in vivo* findings. Similarly, sodium dichromate and other hexavalent chromium compounds were associated with an increased incidence of lung cancer in man^{9,10} and were generally positive in animal assays, by inducing tumours at implant sites¹¹.

Further investigations are needed to elucidate the metabolic pathways leading to conversion of mutagenically active compounds into inactive metabolites. Use of microsomal fractions from various human or rat tissues should be helpful in assessing an appropriate correlation with epidemiological and animal data. The results of an extensive study of hexavalent chromium mutagenicity¹² suggest, for instance, that the metabolic deactivation of the metal by liver preparations is due to reduction to the trivalent form. In contrast to the hexavalent form, trivalent chromium is neither toxic nor mutagenic for *S. typhimurium*¹³ or *Escherichia coli*¹⁴. Interestingly, such conversion apparently also occurs in human erythrocytes, while rat lung and muscle microsomal fractions do not affect the mutagenicity of hexavalent chromium¹².

I thank Drs P. Zanacchi and C. Bennicelli for their assistance.

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Received 14 October; accepted 21 November 1977.

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Biochemical characterisation of stages of hepatocarcinogenesis after a single dose of diethylnitrosamine

CURRENT understanding of the pathogenesis of neoplasia rests principally on the demonstration¹⁻³ that the induction of carcinomas of mouse skin by hydrocarbons can be separated into at least two stages. There is evidence of similar stages in the natural history of carcinogenesis of nonepidermal tissues⁴⁻⁷. For example, Peraino *et al.*⁸ have shown that short term feeding of acetylaminofluorene to rats followed by a long term diet containing 0.05% phenobarbital results in 100% incidence of hepatomas, whereas animals receiving acetylaminofluorene and no phenobarbital developed 10 times fewer hepatocarcinomas. Peraino's procedure and that of Kitagawa *et al.*⁷, who fed rats azo dye followed by phenobarbital, required that the

Table 1 Effect of phenobarbital fed to rats given diethylnitrosamine after partial hepatectomy

Treatment	No. of enzyme-altered foci per cm ² liver at 8 months	Hepatomas
0.05% Phenobarbital (5)	0	0
DEN + PH (6)	3,370 ± 200	0
DEN + PH + 0.05% phenobarbital (8)	18,500 ± 900	7

Rats were partially hepatectomised (PH) and diethylnitrosamine (10 mg per kg) was administered as before⁸. Two months later one group receiving diethylnitrosamine and a control group were fed 0.05% phenobarbital with standard laboratory chow for a subsequent 6 months. The third group continued to receive the chow diet for the remainder of the experiment. At the end of this time animals were killed and sections of the liver were removed and frozen on solid CO₂. Serial sections of the frozen blocks of hepatic tissue were cut and stained consecutively with haematoxylin and eosin and for glucose-6-phosphatase¹⁰, canalicular ATPase⁹ and γ -glutamyl transpeptidase¹². Photographs were taken of each stained section and the number of enzyme-altered foci on each section was determined from tracings using transparent plastic. By appropriate overlaying of the three transparencies, one for each stain, the total number of enzyme altered foci could be calculated by the procedure of Scherer *et al.*²⁰.

carcinogen be fed to the rats for 3-8 weeks. On the other hand, Scherer and Emmelot⁸ have shown that a single large dose of diethylnitrosamine given to rats within 24 h of partial hepatectomy can induce hepatocellular carcinomas, whereas low doses (< 30 mg kg⁻¹), given in the same way, give rise only to small foci of cells deficient in ATPase, comparable with those described earlier^{9,10}. Further, Solt and Farber¹¹ have reported that a single dose of diethylnitrosamine followed by acetylaminofluorene and partial hepatectomy rapidly produces foci containing γ -glutamyl transpeptidase, in contrast to the situation in normal liver which exhibits no histochemical activity of that enzyme. By combining the procedure of Scherer and Emmelot⁸ with that of Peraino *et al.*⁸, we have been able to distinguish clearly between two stages in the genesis of liver cancer in rats.

We used the method of Scherer and Emmelot⁸ followed by the regimen of Peraino *et al.*⁸ (Fig. 1). The results are shown in Table 1. Unlike previous studies^{8,10}, ours involved the scoring of enzyme-altered foci in serial frozen sections by means of stains for three different enzymes: glucose-6-phosphatase¹⁰, canalicular ATPase⁹ and γ -glutamyl transpeptidase¹². In accord with the earlier studies^{8,11}, we found foci exhibiting a deficiency of glucose-6-phosphatase and/or canalicular ATPase as well as a positive stain for γ -glutamyl transpeptidase alone

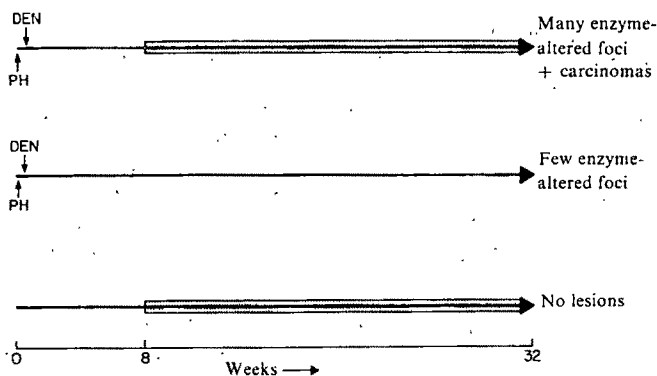


Fig. 1 Schematic representation of the protocol used for the induction of enzyme-altered foci and carcinomas in rats given a single dose of diethylnitrosamine (DEN, 10 mg per kg) by intubation 24 h after partial hepatectomy (PH)⁸. Eight weeks later a group of these animals was placed on a diet containing 0.05% phenobarbital (indicated by shading), while the other group received the same diet containing no drug. A control group was fed 0.05% phenobarbital during the same period. In all experiments female Sprague-Dawley rats weighing 200-220 g were used.

or in combination with a deficiency of one or both of glucose-6-phosphatase and ATPase (Table 2). Phenobarbital alone, even after 6 months, produced no enzyme-altered foci. But when administered for 6 months, after a 2-month interval following partial hepatectomy plus diethylnitrosamine (5–10 mg per kg), this drug caused a six- to eightfold increase of enzyme-altered foci and most animals had hepatocellular carcinomas. Evidence that such enzyme-altered foci represent an early clonal stage in the development of hepatocellular carcinomas has been presented before^{9,13,14}, suggesting that in hepatocarcinogenesis it is possible to identify histochemically the immediate progeny of the putative initiated cells.

On the basis of earlier studies^{15,16}, we established the biochemical phenotype of each enzyme-altered focus using a suitable combination of overlay transparencies traced from photographs of three serial sections, each obtained for one of the enzymes in question. Figure 2 shows a typical composite overlay with different symbols for each area exhibiting one or more enzyme alteration(s). It was prepared from the liver of an animal given diethylnitrosamine (10 mg per kg) 24 h after partial hepatectomy followed by a 6-month dietary regimen of 0.05% phenobarbital. Extremely heterogeneous small foci and two larger foci of microscopic hepatocellular carcinomas can be seen. The phenotypic heterogeneity is analogous to that seen in both primary and transplanted hepatocellular carcinomas in rats¹⁵. Other studies have demonstrated that hepatocellular carcinomas are heterogeneous for both glucose-6-phosphatase¹⁷ and γ -glutamyl transpeptidase¹⁸.

By this technique, we counted the enzyme-altered foci of each of the seven possible phenotypes (Table 2). In animals given diethylnitrosamine alone, more than 75% of all foci exhibited only a single enzyme alteration with roughly equal frequency. In contrast, 45% of foci in the livers of animals given carcinogen followed by phenobarbital in their diet exhibited γ -glutamyl transpeptidase whereas 30% were equally distributed between those exhibiting ATPase deficiency alone and those exhibiting this deficiency in the presence of γ -glutamyl transpeptidase. The relative proportion of foci exhibiting defects of all three enzymes increased more than twice over that in the animals given the carcinogen only.



Fig. 2 Biochemical phenotypes of enzyme-altered foci in three serial sections of liver from a rat given a single dose (10 mg kg⁻¹) of diethylnitrosamine 24 h after partial hepatectomy followed 2 months later by feeding 0.05% phenobarbital for a subsequent 6 months. Each area is outlined to represent the enzyme alteration exhibited in that region as follows: 0-0-0, glucose-6-phosphatase-deficient areas; - - - - -, ATPase-deficient area; γ -glutamyl transpeptidase present in area. In areas exhibiting more than one enzyme alteration, composite and/or parallel symbols are used. The figure was obtained by overlaying three separate transparencies after tracing on each of the outlines of areas exhibiting one enzyme alteration. Although in some areas not all cells in the focus exhibited the same phenotype, the area was scored as having the phenotype (Table 2) of most of the cells therein.

The mechanism by which the phenotypic heterogeneity of the foci is produced is not clear. In hepatocellular carcinomas this heterogeneity has been suggested to result from alterations in messenger RNA template stability¹⁵. If the phenotypic heterogeneity evident in enzyme-altered foci, the earliest discernible putative precursor of the hepatocellular carcinoma, reflects the phenotypes of the fully developed hepatocellular carcinoma, one may suggest that such biochemical changes

Table 2 Number and phenotypes of enzyme-altered foci per g of liver

	GP, AP, GT	GP, GT	GP, AP	AP, GT	AP	GT	GP	Total
DEN + PH	120±80 (3.6)	100±50 (3.0)	300±170 (8.9)	170±80 (5.0)	930±400 (27.6)	750±250 (22.3)	1,000±400 (29.7)	3,370±200 (100)
DEN + PH + 0.05% phenobarbital	1,450±300 (8.1)	300±100 (1.7)	700±250 (3.9)	2,700±700 (15.1)	2,700±700 (15.1)	8,700±1,100 (44.8)	2,000±500 (11.2)	18,550±900 (100)

GP, glucose-6-phosphatase negative; AP, canalicular ATPase negative; GT, γ -glutamyl transpeptidase positive; DEN, diethylnitrosamine; PH, partial hepatectomy.

Numbers in parentheses represent the percentage distribution of the foci in these conditions.

Five rats given DEN + PH alone and eight rats given DEN + PH + phenobarbital were used for these calculations. See text and Table 1 for further details.

These results show that two distinct stages can occur in hepatocarcinogenesis in analogy to that seen in skin. It is not clear how the promoting agent increases the number of enzyme altered foci; however, this system may be the first in which the immediate progeny of the putative initiated cells have been identified. Possibly, promoting agents not only cause the biological expression of malignancy but also stimulate the replication of dormant initiated cells, which in the absence of the promoter would not have proliferated. If each enzyme-altered focus is a clone derived from such a cell¹⁴, approximately one in 10⁴–10⁵ cells in the liver seem to be initiated by diethylnitrosamine as expressed after feeding with phenobarbital.

The production of enzyme altered foci in the liver may be a general result of hepatocarcinogen administration. Studies from our laboratory (unpublished) as well as those of Pugh and Goldfarb¹⁹ have demonstrated enzyme-altered foci following the administration of azo dyes and acetylaminofluorene to rats.

occur at or shortly after the process of initiation and remain relatively constant throughout the development of a malignant tumour from an initiated cell population.

This work was supported in part by a grant from the NCI (CA-07175).

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Received 1 August; accepted 2 December 1977.

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Myeloid colony-forming cells express human B lymphocyte antigens

STUDIES in experimental animals suggest that lymphoid and myeloid cells share a common stem-cell precursor¹, but human lymphoid-myeloid stem cells have been difficult to document. Indirect evidence for such cells has come from recent observations that human B lymphocyte antigens occur not only on B lymphocytes but also on monocytes and leukaemic myeloblasts²⁻⁶. If normal immature myeloid cells, like leukaemic myeloblasts, share a common differentiation antigen with B lymphocytes, it would seem even more likely that myeloid cells and B lymphocytes share a common stem-cell precursor. We have therefore compared the ability of bone marrow cells to form macrophage-granulocyte colonies *in vitro* after complement-mediated lysis of cells expressing either human B-lymphocyte antigens (HLA) or human T-lymphocyte antigens (HTLA). Our results show that macrophage-granulocyte colony-forming cells (CFU-C) express HBLA but not HTLA antigens.

Ficoll-Hypaque separated mononuclear cells were obtained from bone marrows of children with acute leukaemia or lymphoma in remission. These cell suspensions were depleted of HBLA⁺ or HTLA⁺ cells by treatment with rabbit anti-HBLA or anti-HTLA plus complement (the preparation and specificity of these antisera have been described in detail previously⁷). The viable cells remaining were incubated in methylcellulose with leukocyte-conditioned medium and examined for macrophage-granulocyte colonies after 10 d in culture.

The results of experiments using bone marrow cells from four different subjects are shown in Fig. 1. In every case, compared with control cells preincubated with normal rabbit serum + C, incubation of the marrow cells with anti-HBLA + C before culture resulted in a marked reduction in numbers of colonies formed on day 10. Incubation of cells with anti-HTLA + C, however, resulted, if anything, in an increase in colony numbers on day 10 of culture. Thus the myeloid precursor cells capable of forming macrophage-granulocyte colonies *in vitro* seem to express HBLA but not HTLA antigens. We have been unable to detect HBLA antigens by immunofluorescence or complement-dependent cytotoxicity on mature peripheral blood granulocytes (J.K., unpublished observations). These findings are consistent with recent observations by Ross *et al.*⁸ that rabbit antisera to purified lymphocyte membrane components detect B-lymphocyte antigens on normal bone marrow myeloblasts and myelocytes but not on metamyelocytes, band forms or mature granulocytes.

That B lymphocytes and immature myeloid cells share common antigens supports the concept that lymphoid and myeloid cells arise from a common stem-cell precursor. The fact, however, that T lymphocytes and even relatively im-

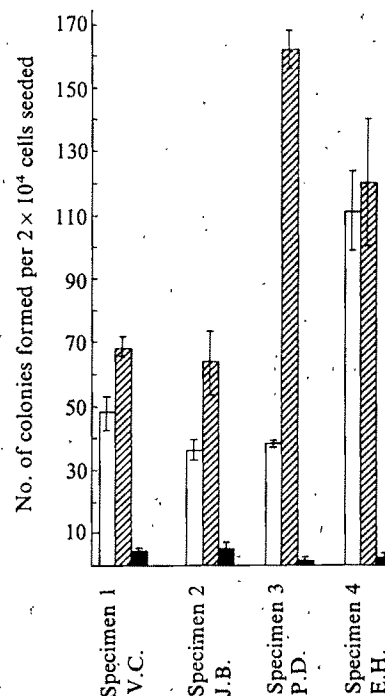


Fig. 1 Effect of complement-mediated lysis of HBLA⁺ or HTLA⁺ bone marrow cells on *in vitro* macrophage-granulocyte colony formation. Suspensions containing 10^7 cells ml^{-1} diluent (RPMI) 1640 (with 20% foetal calf serum) were incubated for 60 min at 37°C with equal volumes of 1:10 diluted heat-inactivated rabbit anti-HTLA or anti-HBLA and complement (fresh normal rabbit serum). Control cells were incubated with heat-inactivated normal rabbit serum and complement. After determining the percentage viability by Trypan blue exclusion, the cells were washed three times and the cell concentration adjusted to 1×10^5 viable cells ml^{-1} alpha-media. The cells were cultured for 10 d in 1% methylcellulose in quadruplicate plates as described previously¹³ using leukocyte-conditioned medium at a 20% concentration (v/v) as the source of stimulators. Open columns, normal rabbit serum + complement; hatched columns, anti-HTLA + complement; solid columns, anti-HBLA + complement.

mature T-cell precursors⁸, lack HBLA antigens raises the question of whether the stem cells for T lymphocytes are distinct from the stem-cell precursors of B lymphocytes and myeloid cells. Recent observations that terminal transferase (TdT), an enzyme normally found only in thymocytes and a small population of immature bone marrow cells⁹⁻¹¹, occurs in HBLA⁺ as well as HTLA⁺ leukaemic lymphoblasts (J.K., unpublished data) and in some leukaemic myeloblasts¹² mitigates against this possibility and suggests instead that T cells, B cells and myeloid cells all share a TdT⁺ stem-cell precursor. From the present findings it would seem likely that such a stem cell also expresses HBLA antigens which are lost at a prethymic stage of T-cell differentiation, just as they are lost at a metamyelocyte stage of granulocyte differentiation. Attempts to test this model of leukopoiesis further would be facilitated by the availability of improved methods for the detection and isolation of human lymphoid-myeloid stem cells. Antisera to HBLA antigens could be useful tools in such endeavours.

This research was supported by US Public Health Service, grants from the National Institutes of Health and by the Bonnie Baker Fund.

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Stable variants affecting B cell alloantigens in human lymphoid cells

THE human major histocompatibility complex (MHC) is defined by four loci: *HLA-A*, *B* and *C* are serologically defined and *HLA-D* was initially defined by the mixed lymphocyte reaction (MLR)¹. There is now evidence that a group of HLA-linked, serologically defined B cell-specific alloantigens are the gene products of either the *D* locus itself or of closely linked genes^{2,3}. Strong associations are seen between alleles of *HLA-D* and the B cell-specific genotypes, and B cell antisera inhibit the MLR^{4,5}. The *D*-associated antigens differ from the products of *HLA-A*, *B* and *C* in chemical structure and in tissue distribution⁶. Human B-cell alloantigens resemble Ia antigens in mice. Analysis of the relationships among Ia antigens, MLC loci and other functions mapping in the I region is limited in both humans and mice by the availability of recombinant individuals⁷. Because such recombinants are rare, we have begun an analysis of this region by mutation⁸, using immunoselection to isolate mutants defective for MHC gene products in human lymphoid cell lines. We describe here the first Ia variants to be isolated and characterised in any species.

The B cell-specific alloantibodies used for selection of the variants were isolated from antiserum 0800 (Associated Bio-medical), which also contained antibodies against HLA-B8. This antiserum reacted with some non-B-8 cells when tested on B-cell lymphoid lines. UM21, a non-B-8 cell line which reacted with 0800, was used to absorb the non-HLA-8 antibody, which was then eluted from the cells by incubation for 30 min at 0 °C in glycine-HCl-saline buffer pH 3.0, 0.5 M. This material is referred to as 0800 eluate (0800E). 0800E has the properties of B cell antibody⁹: first, in individuals reactive with 0800E, 10-15% of PBLs were labelled by indirect immunofluorescence; second, after separation of lymphocyte subpopulations from reactive individuals by anti-F(ab) column or sheep red blood cell (SRBC) rosettes, the B cell-enriched fraction gave 60-65%

Table 1 2×2 Comparison of 0800 eluate and WIA1 antisera of 7th Workshop

	WIA1	
	+	-
0800 Eluate +	4	0
-	0	9

positive cells and the T-cell fraction <1% positive cells; third, fowl anti-β2 microglobulin, which blocks the cytotoxic activity of alloantisera to HLA-A, B and C but not of B cell-specific antibody¹⁰, reduced the titre of 4 A and B alloantisera on sensitive target cells, but did not alter the cytotoxicity of 0800E. The 0800E reacted with 4 of 17 B-cell lines and neither of 2 T-cell lines. Thirteen of the B-cell lines were also tested with 169 B-cell alloantisera of the 7th Histocompatibility Workshop (7WS) panel¹¹, and 0800E showed correlation with the antiserum cluster WIA1 (Table 1).

Table 2 Cytotoxicity titres of B cell-specific sera, HLA sera and anti-β2-microglobulin sera

	Wild type progenitor T5-1	Variants (6.1.6) (8.1.6)
B cell-specific alloantisera		
0800 eluate	4	< 1
277	2	< 1
HLA-A and B alloantisera*		
HLA-B8		
0800 absorbed	16	8
CAMPBELL	4-8	4
CHAYRA	2	2
HLA-B27	16-32	16
HLA-A2 (R3 absorbed rabbit heterosera)	8-16	8
HLA-A1		
SHILEY	4-8	4-8
B cell-specific heterosera		
814	128	8-16
Anti-55	64-128	4-8
Anti-23-30	400-800	< 1
R20 (anti-T5-1, absorbed with 6.1.6)	16-32	< 1
Anti-β2 microglobulin (rabbit)	256-512	256

All antisera were tested for cytotoxicity by the Terasaki method. Doubling dilutions were made in human AB sera (except for anti-β2-microglobulin where RPMI 1640 was used) and pooled rabbit complement which had been absorbed with T5-1 cells was used. The titre of a serum on a cell line is the highest dilution giving 50% kill.

*Alloantisera except where otherwise noted; HLA antisera for HLA-A2, B8 and B27 are described elsewhere¹².

The cell line used in the selection was T5-1, which has been used in previous studies on HLA variants¹². T5-1 has a pseudo-diploid karyotype and HLA haplotypes *HLA-A1*, *B8* and *HLA-A2*, *B27*, *CW1*. With respect to B cell-specific alloantigens, the cell line reacts with all sera in clusters defining WIA1 and WIA3, which seem to be alleles of a single locus¹¹.

Before selection, T5-1 was mutagenised as described previously¹¹ to increase the frequency of variants. Logarithmically growing cells were suspended in either 4 μg ml⁻¹ ICR-191 or 50-100 μg ml⁻¹ ethylmethanesulphonate (EMS) for 24 h. After washing twice and resuspending in fresh medium, the cells were cultured until the viability was ≥80% as measured by Trypan blue exclusion. For the selection of variants, 2.5-5.0×10⁵ cells were incubated in 0.1 ml antiserum for 45 min at 22 °C, centrifuged and then exposed to pooled absorbed R C' in excess for 90 min at 37 °C, washed and then plated in soft agarose⁸. Surviving clones were isolated approximately 14 d later and tested with 0800E. Among a total 1×10⁶ cells treated with ICR-191 and selected against, a single 0800E-resistant clone was found (6.1.6). Another aliquot of the same ICR-191-treated cells gave 22 HLA-B27 variants for every 10⁶ cells selected. Two 0800E-resistant clones (8.1.6 and 8.2.6) were recovered from 2.5×10⁵ EMS-treated cells; another aliquot of these cells gave 24 B27 variants per 2.5×10⁵ cells.

Resistant clones 6.1.6 and 8.1.6 were tested with the selecting antiserum at least five times over a period of 6 months of cultivation in normal medium (a total of ≥80 population doublings) and the phenotype was stable. Cytotoxicity assays with a panel of A and B antisera (Table 2) showed that the variants were essentially unaltered in expression of HLA-1, 2, 27 and 8.

To define the nature of the antigenic loss further, the variants were tested with the 169 B cell-specific (Ia) 7WS alloantisera (Table 3) and with several rabbit sera prepared against partially purified human Ia. Variant 6.1.6 has lost reactivity with both WIA1 (4/4 sera) and WIA3 (5/5 sera) and, in fact showed no reactivity with any of the 44 sera that defined antigenic clusters. Of 58 sera that reacted with T5-1, only one weak reaction was retained, and this was with a serum which

also reacted with a T-cell line. In addition to the loss of alloantigenic determinants, 6.1.6 seems to have lost the constant portions of the Ia molecule as indicated by nonreactivity with anti-P23, 30 (ref. 6), a heteroantiserum prepared against papain solubilised, purified B cell-specific glycoprotein, which has a titre of 200–400 against T5-1. Antiserum 814¹³ and anti-55 (A. Fuks, unpublished) are antisera against immunogens prepared by detergent solubilisation and partial purification of the alloantigen-bearing glycoprotein dimer from two different B-cell lines and both showed reductions in titre (from 128 to 8–16 and from 64–128 to 4–8 respectively (Table 2)). In each case, this residual reactivity was approximately equal to that seen against the T-cell line HSB-2.

To demonstrate that the nonreactivity of 6.1.6 with anti-23-30 was not due to nonspecific masking of the antigen, deoxycholate was used to lyse cells and solubilise membrane glycoproteins from T5-1 and 6.1.6, and the lysates were tested for antibody-binding according to the method of Reif^{14,15}. In three experiments wild-type cells gave an average *g* value, that is, number of cells to absorb one half of cytotoxic antibody, equal to 2.1×10^3 cells/ λ , but in each experiment lysates from 6.1.6 bound no antibody at cell levels equivalent to 1.68×10^5 cells. Thus the amount of antigen in 6.1.6 is $< 1/80$ of that of the wild type.

Further evidence that 6.1.6 had undergone loss of the constant portion of Ia came from absorption experiments in which rabbit antiserum raised against T5-1 was absorbed with 6.1.6. The absorbed antiserum was tested for reactivity with cell lines and on B and T cells from peripheral blood (Table 4). This absorbed antiserum should be specific for determinants missing in the variant¹⁸. The absorbed antiserum reacted with T5-1 and with all B-lymphoid lines with approximately equal cytotoxicity titres but with neither of the two

T cell lines. Moreover, the absorbed antiserum reacted with titres of 8 to 16 on separated B lymphocytes from three individuals but it gave no cytotoxicity with the T lymphocytes. Thus heteroantiserum made against T5-1 and absorbed with 6.1.6 recognises determinants present on B cells of all 18 individuals tested but not on T cells, consistent with the interpretation that 6.1.6 had lost the common portion of Ia.

The variant 8.1.6, also selected as resistant to the 0800E, has a more limited lesion; it was indistinguishable from wild type with the heterosera described above. Tested with the 7WS panel, it showed loss of reactivity for WIA1 (4/4 sera) but unaltered reactivity for WIA3 (5/5 sera) (Table 3). The variant thus has an alteration in the WIA1 allele. Among the sera other than those mono- and multispecific sera recognising defined specificities, seven sera (our designation UW-X) react with T5-1 but not with 8.1.6. These 'UW-X' sera also react with WIA1 and WIA2 among our B-cell lines. The UW-X sera may represent polyspecific sera (which react only with IA-1 in T5-1) or they may detect a public specificity which is shared by IA1, -2, and -6 and which is included in the 8.1.6 lesion.

Table 4 Cytotoxicity titres of rabbit heterosera prepared against T5-1 (progenitor) and absorbed with variants

		Anti-T5-1 absorbed with 6.1.6		absorbed with 8.1.6
		6.1.6	8.1.6	
T5-1	10.2.5	16–32	4	
	6.1.6	<1	—	
	8.1.6	16–32	4	
15 Unrelated B cell lymphoid lines*		4–8	—	
T cell lines				
	8402	<1	—	
	HSB-2	<1	—	
Peripheral blood lymphocytes (3 donors)				
	B-cell fraction†	8–16	—	
	T-cell fraction†	<1	—	

Fixed volumes of antisera were absorbed with varying numbers of variant cells to attempt to find a minimal number which would remove all cytotoxicity against the variant. Data shown are for antisera absorbed with 1×10^6 cells λ^{-1} .

*The other B lymphoid lines tested were 8866, BREL, ORI, BRISTOL 8, UM-21, DAUDI, HUP-2, SB-1, SC-LA, SC-TA, SC-JB, SC-BM, SC-CA, SC-AM, RAJI, and two T-cell lines HSB-2, 8402.

†Peripheral blood lymphocytes were fractionated on an anti-F(ab) column. B-cell fractions contained 94–96% s Ig-positive cells and T-cell fractions contained 1–6 s Ig positive cells. — = not done.

The two variants, both selected with the same antiserum, thus have different properties from one another. This is consistent with evidence previously obtained^{11,17} that the antiserum selects pre-existing variants rather than inducing modulation of the reactive antigen. 8.1.6 seems to have lost the WIA1 determinant but has retained the WIA3 determinant as well as the 'common portion' of the molecule, as determined by reactivity with the heterosera. In this respect 8.1.6 seems analogous to most of the A and B variants previously isolated. 6.1.6 is altered not only for the WIA1 alloantigenic determinant selected against but also does not express the allelic determinant WIA3 nor any 'constant portion' of the HLA-linked B cell specific glycoprotein as judged by two independent lines of evidence; first, the absence of antibody-binding from anti-23, 30¹⁸ by 6.1.6 whole cells or lysates, and, second, rabbit anti-T5-1 absorbed with 6.1.6 reacts with all B cells tested but with none of the T cells. 6.1.6 could be explained by a single mutation in a regulatory locus or by mutations in two structural genes or by an epigenetic event. Although 6.1.6 lacks both WIA1 and WIA3, the phenotype is unlikely to be the result of two mutations, one in each of the genes carrying these determinants because the frequency of such an event would be the square of the single event and no single mutants were observed among the ICR-191-tested cells. If 6.1.6 is due to a change in a

Table 3 IA typing of T5-1 and variants by 7WS panel

WIA antigen	Serum	Cytotoxicity scores*		
		T5-1	6.1.6	8.1.6
1	7W009	3.5	—	—
	7W042	4.0	—	—
	7W120	4.0	—	—
	7W128	3.5	—	—
	7W152	4.0	—	—
3	7W024	3.5	—	4.0
	7W026	4.0	—	4.0
	7W028	4.0	—	4.0
	7W035	4.0	—	4.0
	7W133	2.0†	—	4.0
6+3	7W075	4.0	—	4.0
	7W111	4.0	—	4.0
	7W157	4.0	—	4.0
6+2(+1)	7W046	4.0	—	†3.0
	7W144	4.0	—	—
	7W179	3.0	—	—
UW-X	7W004	4.0	—	—
	7W010	4.0	—	—
	7W066	4.0	—	—
	7W105	4.0	—	—
	7W114	4.0	—	—
	7W150	3.0	—	—
	7W156	4.0	—	—

The sera of 7WS have been grouped into clusters defining eight groups. Results are shown for sera in clusters WIA1, WIA3, WIA6+3, WIA6+2+1 and a group of sera UW-X. Not shown are results for those clusters which gave a negative reaction pattern on both T5-1 and its variants. (Clusters WIA2, 4, 5, 7, 8, WIA A, IA4×7.) UW-X is the group of sera which react with T5-1 and are altered in the variant 8.1.6. (Analysis of UW-X on normal unrelated cell lines showed caucasian derived lines with either WIA1 or WIA2 were reactive; also reactive were two non-caucasian-derived lines, Daudi (African Black) and SJ-AH (Japanese), which are not WIA1 or WIA2. For discussion of nature of UW-X, see text.)

*Methods for cytotoxicity tests as in Table 2. Scores are means of two tests of undiluted antibody; values 2, 3, 4 indicate consistent positive reactions of increasing strength. —, Score ≤ 1.5 .

†Cases in which difference in score between replicates ≥ 2 .

regulatory locus, it is not associated with a switch to expression of a different alloantigenic allele detectable by alloantisera or by absorbed rabbit antisera. The possibility that 6.1.6 arose by an epigenetic event is an open one unlike previously described variants for HLA-A and B where there is a preponderance of evidence for the variants being the products of single gene mutations^{8,11,17}. Regardless of the nature of the 6.1.6 lesion, it is clear from the unchanged expression of HLA-A and B that it does not affect these other MHC-coded membrane glycoproteins.

The typing of alloantigenic variants such as 8.1.6, delineates Ia specificities in a manner which is independent from the conventional delineation by pedigree analysis. If the human Ia system resembles murine Ia in being composed of several tightly linked loci, the mutational approach should make this apparent by distinguishing the products of independent loci whereas pedigree analysis would require the discovery of rare recombinants. In the case of 8.1.6 the fact that reactions with five WIA1 sera were altered coordinately suggests that these sera probably are all reactive with the products of the same single locus. Ia variants should also be useful in exploring other questions such as the nature of the determinants which stimulate in the MLC and the regulation of Ia expression.

We are grateful for gifts of antisera from R. Winchester (heterosera 814 and allosera 277); A. Fuks, J. Kaufman and J. Strominger (heterosera anti-55 and anti-23, 30); P. Goodfellow (fowl anti- β 2m sera). We thank J. Sullivan for pairs of T and B cells, J. Callis for cell analysis by microfluorimetry, and Penny Bowman and Carl Soderland for technical assistance. This work was supported by US NPH (grants GM-15883 and HD-05961).

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Received 17 October; accepted 12 December 1977.

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Production *in vitro* of murine antibody to a human histocompatibility alloantigen

AN *in vitro* culture system from our laboratory which may be used to generate homogeneous antibodies to human cell surface antigens has been recently described¹. Using this system, we have obtained series of antibodies which discriminate between closely related human cells¹ and cell lines². The present report defines more precisely the potential of this system by showing how it may be used to obtain antibodies to a human cell surface antigen of well-defined specificity, the histocompatibility alloantigen HLA-A2.

The mouse spleen fragment culture system involves injecting limited numbers of donor spleen cells, with immunogen, into irradiated, syngeneic recipients; the recipient spleens are diced

into 1-mm³ fragments, which are cultured individually. The dose of donor cells is adjusted so that each fragment receives at most a few antibody-forming cell precursors which recognise the immunogen; these precursors proliferate during the culture period, and the antibody produced by the daughter cells is secreted into the culture medium^{1,3}. In the present study, the A2⁺ human lymphoblastoid cell line 8866 (ref. 4) was used as the immunogen, and the culture fluids from 167 fragments were studied. A radioimmunoassay^{2,5} was used to measure the antibody-binding activity of each fluid against both 8866 and an A2⁻ variant of that line, subline 1-2. This variant was isolated by immunoselection with an allogeneic anti-A2 antiserum, and lacks both cell surface and internal A2^{1,6,7}. We found that 141/167 fluids contained antibody to 8866, and all but three of these showed identical binding to that cell and its A2⁻ variant. The three remaining fluids, B9, F4 and H4, showed significantly greater binding to the A2⁺ cell (Fig. 1a-c).

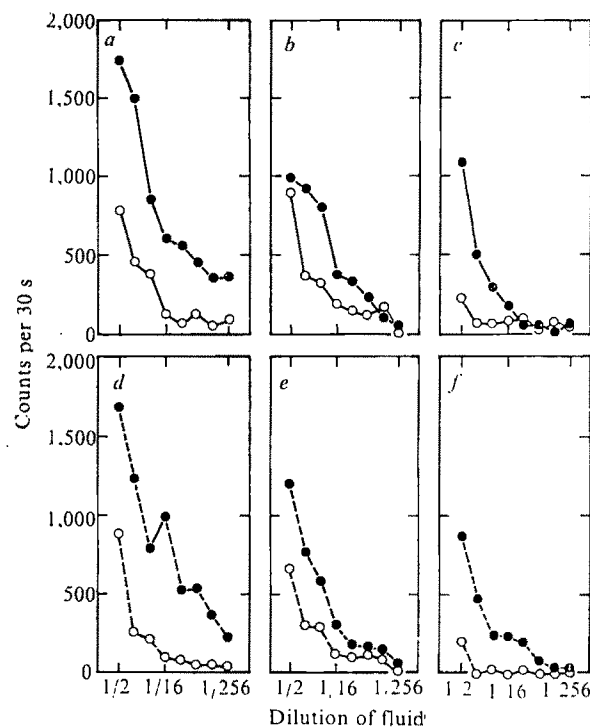


Fig. 1 Binding of discriminator culture fluids to A2⁺ and A2⁻ cell lines. Antibody binding activity in culture fluids was assessed by radioimmunoassay. Generation of culture fluids: 10⁷ viable donor spleen cells were given intravenously to each of four lethally irradiated recipient mice; a few minutes later, 10⁷ 8866 cells were given intravenously to each recipient; 18 h later, recipient spleens were diced into 1 mm³ fragments and the fragments were cultured individually in 96-well plastic plates for 4 weeks, during which time the culture fluids were collected and replaced with fresh medium every 3-4 d. Donor mice were (BALB/c × C57BL/6) F1 mice that had received intraperitoneal injections of 10⁷ 8866 cells 15, 9, and 6 weeks before use. Recipient mice were F1 mice that had received a single intraperitoneal injection of 10⁷ 8866 cells 5 weeks before use. Radioimmunoassay: 25 μ l of culture fluid was incubated for 2 h at room temperature with 2 times 10⁵ glutaraldehyde-fixed target cells; target cells were washed extensively, and then incubated with 10 μ l ¹²⁵I-goat anti-mouse κ (30,000 c.p.m./10 μ l) (ref. 2) for 18 h at 4°C; the cell pellets were washed extensively, dried, and counted in a γ counter. Graphs show (counts bound with test fluid) - (mean of counts bound with eight control fluids). Control fluids were generated by culturing fragments from spleens which had received neither donor cells nor antigen. Mean control binding for each cell type: 8866, 188; 1-2, 214; T5-1, 211; 6.6.5, 202. a-c, Binding activity to 8866 (●) and variant 1-2 (○); d-f, binding activity to T5-1 (●) and variant 6.6.5 (○). Fluids F4 and B9: fluids collected after 7 and 10 d of culture were pooled for assay; a later collection (after 14 d) showed similar discrimination between A2⁺ and A2⁻ cell lines (not shown). Fluid H4: assays were performed on 14-d collection; an earlier collection also showed discrimination, but less overall activity (not shown). a and d, Fluid B9; b and e, fluid F4; c and f, fluid H4.

The specificity of the three discriminator fluids was verified in two ways; first, the radioimmunoassay was used to show that each of the three fluids also discriminated between a second A2⁺ cell line, T5-1, and an A2⁻ variant of that line, subline 6.6.5 (ref. 8) (Fig. 1*d-f*); second, a cytotoxic assay was used to assess the specificity of the fluids with respect to normal human peripheral blood lymphocytes (PBL). Each of the three fluids was cytotoxic for 2/2 A2⁺, A28⁻ PBL, and negative for 2/2 A2⁻, A28⁻ PBL (data not shown). Fluids B9 and F4 were also tested in a more extensive typing panel (Table 1), which confirmed that both fluids recognised A2⁺ cells, as well as cells bearing the highly cross-reactive⁹ specificity A28. Neither fluid

Table 1 Cytotoxic activity of discriminator fluids against normal human PBL.

Test cells	Fluid B9		Fluid F4	
	Sample 1*	Sample 2†	Sample 1*	Sample 2†
A2 ⁺ , A28 ⁻	13/13	14/14	12/13	2/2
A2 ⁻ , A28 ⁺	6/7	12/12	5/7	2/2
A2 ⁻ , A28 ⁻	0/10‡	0/19§	0/10‡	0/2

Cytotoxic testing was performed in plastic typing trays, using PBL labelled with fluorescein diacetate (FDA): 2,500 cells in 1 µl were incubated with 1 µl of culture fluid for 30 min at room temperature, after which 5 µl of fresh frozen rabbit serum was added; cell viability was determined after an additional room temperature incubation for 2h 30 min. Table shows the ratio of target cells showing strong positive reactions to target cells tested.

*Fluids collected after 7 and 10 d of culture were pooled for assay.

†Assay performed on fluid collected after 14 d of culture.

‡All specificities known to be present on the immunising cell⁴ were represented.

§All known HLA-A and HLA-B specificities and most Aw and Bw specificities were represented.

was cytotoxic for any cell lacking both of these specificities; in particular, neither fluid recognised any of the other HLA specificities known to be present on the immunising cell⁴.

In order to test whether the A2 and A28 reactivities were separable by absorption, serial dilutions of fluids B9 and F4 were absorbed with constant numbers of each of three types of PBL: A2⁺ cells, A28⁺ cells and, as a control, A2⁻, A28⁻ cells. The residual cytotoxic activity against both A2⁺ and A28⁺ target cells was then measured (Table 2). For each fluid, absorption with a constant number of A2⁺ cells significantly reduced the cytotoxic activity against both A2⁺ and A28⁺ target cells. Similarly, absorption with A28⁺ cells significantly reduced the cytotoxic activity against both types of target cells. These results suggest that fluids B9 and F4 contain antibody which is directed against a cross-reactive determinant present on both A2⁺ and A28⁺ cells. Although the cross-reactivity between these cells is well established, its molecular basis is not yet fully understood⁹. Antibodies such as those described here should prove valuable tools in analysing these problems. Figure 1 shows that fluids B9 and F4 each showed some binding activity to the A2⁻ cell lines. Because both A2⁻ variants have been shown to lack surface A2 and A28 (refs 4 and 6, and D. P., unpublished results) we attribute this binding to the production of one or more additional antibodies by these fragments. Also, because no extra activities were seen in the extended typing panels, these antibodies may not recognise (or not be cytotoxic for) antigens found on normal human PBL. These points will be explored more fully in a later report (L. A. L. *et al.*, in preparation).

Many laboratories have produced xenogeneic anti-HLA antisera^{10,11}. Our approach differs from previous work in that murine anti-HLA antibodies were produced *in vitro*, and that, even though a whole cell was used as the immunogen, we obtained antibodies which, without further purification, were specific with respect to HLA. The value of *in vitro* cloning techniques for the production of homogeneous antibodies to cell

Table 2 Absorption of discriminator fluids

Absorbing cell	Cytotoxic titre remaining after absorption			
	Fluid B9*		Fluid F4†	
	Target cells A2 ⁺ , A28 ⁻ A2 ⁻ , A28 ⁺		Target cells A2 ⁺ , A28 ⁻ A2 ⁻ , A28 ⁺	
B.C. (A2 ⁻ , A28 ⁻)	32	≥ 64	4	2
K.W. (A2 ⁻ , A28 ⁻)	—	—‡	4	2
J.S. (A2 ⁺ , A28 ⁻)	4	4	0	0
C.A. (A2 ⁺ , A28 ⁻)	4	4	1	1
B.S. (A2 ⁻ , A28 ⁺)	8	8	1	0
L.B. (A2 ⁻ , A28 ⁺)	8	8	1	0

One µl (45,000) absorbing cells were incubated with 1 µl culture fluid for 1 h at 37 °C, after which 1 µl (2,500) FDA-labelled target cells were added, and the cytotoxic test carried out as in Table 1.

*Day 14 collection. Pre-absorption titres: A2⁺, A2⁻ target, ≥ 64; A2⁻, A28⁺ target, ≥ 64.

†Day 7+10 collection. Pre-absorption titres: A2⁺, A2⁻ target, 4; A2⁻, A28⁺ target, not done.

‡Not done.

surface antigens is widely recognised^{1,2,12,13}. This report demonstrates the ability of the mouse to recognise a well-defined human cell-surface alloantigen, and describes conditions under which there is a reasonable probability of finding antibodies specific with respect to HLA. In addition, we have shown the value of using HLA⁻ variant cell lines⁸ for the rapid identification of these antibodies. The mouse spleen fragment culture system, as used here, provides a versatile way of obtaining antibodies to human cell surface antigens in quantities suitable for analytical work^{1-3,14}. This system also provides a way of enriching for antibody-forming cells of a desired specificity; these may then be expanded by techniques such as transformation or somatic cell hybridisation into a more permanent source of the desired antibody.

This work was supported by funds from the USPHS and the American Cancer Society. L. A. L. was supported by an NIAID Immunobiology Postdoctoral Fellowship.

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Received 13 October; accepted 28 November 1977.

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Coupling PPD to tumour cells enhances their antigenicity in BCG-primed mice

STUDIES of cell cooperation and of the carrier effect in the induction of antibody formation and in delayed hypersensitivity¹ can predict, and have indeed shown²⁻⁴, that combining an antigenic determinant which evokes a powerful T cell response with a weak antigen should greatly enhance the immunogenicity of the latter by providing 'antigenic help'.

Tuberculin (purified protein derivative, PPD) is a particularly effective carrier antigen. Long lasting and powerful T-cell reactivity (or delayed hypersensitivity) to tuberculin can readily be induced by infection with *Mycobacterium tuberculosis* vaccine (BCG). On the other hand, PPD itself does not induce delayed hypersensitivity but elicits reactions in the sensitised host. This property allows the effects of 'antigenic help' to be distinguished from changes in immunogenicity produced by chemical modification of the antigen simply by comparing the effects in BCG-primed and normal animals. Furthermore, there is little, if any, antibody response to PPD itself. Previous work from our laboratory has shown that PPD acts as a powerful carrier for the raising of anti-NIP antibodies in BCG-positive but not in BCG-negative hosts⁵. Immunising BCG-positive animals with PPD coupled to a weak protein antigen (for example, $\beta 2$ microglobulin) similarly enhances markedly the amount of anti- $\beta 2$ microglobulin that can be made (P. J. Lachmann, unpublished observations). We here report on the use of chemical modification with tuberculin for producing 'antigenic help' in raising immunity to the tumour specific transplantation antigens (TSTAs) of methylcholanthrene-induced sarcomas in syngeneic mice.

The tumours used were induced in female C57B1/10 ScSn (B10) mice by the subcutaneous implantation of 0.5 μ g 3-methylcholanthrene and were selected for immunogenicity as previously described⁶. The two tumours used in this study (designated MC 6A and MC 6B) were produced in a single mouse and were found to be non-crossreactive in a rejection assay. In initial experiments, tuberculin (PPD, Central Veterinary Laboratories, Weybridge, Surrey) was coupled directly to the irradiated tumour cells using redistilled glutaraldehyde at a final concentration of 0.005 M for

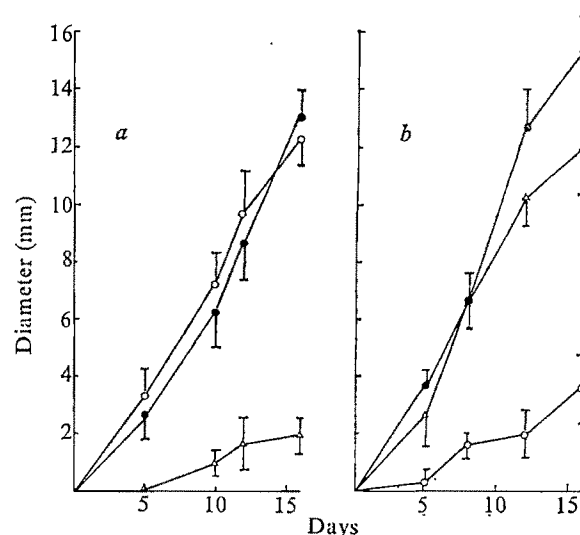


Fig. 1 Specificity of protection by cells coupled to Con A-PPD. Groups of three mice were immunised with either MC 6A (Δ) or MC 6B (\circ) cells coupled to PPD as in the legend to Table 1. Animals were challenged with 2×10^5 cells of either MC 6A (a) or MC 6B (b). Groups of unimmunised mice (\bullet) were similarly challenged. Mean tumour diameters were measured at 3-d intervals. Error bars represent \pm s.e.m.

2 h at room temperature. This procedure, however, seemed to cause damage to the tumour specific antigens and for this reason an alternative strategy was employed. The tuberculin was coupled to concanavalin A (Con A) using glutaraldehyde as above. For this purpose 10 mg of Con A (Calbiochem) in 1 ml was mixed with 10 mg of 125 I trace-labelled PPD also in 1 ml and redistilled glutaraldehyde was added to a final concentration of 0.006 M. After 2 h at room temperature the reaction was stopped by the addition of 1/10 volume of molar lysine. The mixture was then fractionated by affinity chromatography on G75 Sephadex. The material passing through this column (more than 95% of the tuberculin added) was rejected and then Con A and Con A-PPD were eluted from the column with 0.1 M α -methylglucose. The Con A concentration in the eluted peak was measured by the agglutination titre on guinea pig erythrocytes and the PPD by counting the 125 I. This isolated Con A-PPD was dialysed to remove sugar and added to irradiated ($10,000$ R) tumour cells (the equivalent of 20 μ g PPD was added to 10^8 cells). After incubation at room temperature for one hour, the cells were washed three times and the amount of bound 125 I determined by counting the washed cells in a γ counter; 25–50% of the added counts bound to the cells. The amount offered to the cells was chosen to give between 2 and 5×10^6 molecules of PPD per cell.

Groups of animals were immunised as described in Table 1. All the immunisations were performed on both BCG-primed and control (BCG-negative) animals to allow the role of delayed hypersensitivity to tuberculin to be assessed. Immunisation with tumour cells mixed with tuberculin was compared to immunisation with PPD-coupled cells. This allows the effect of injecting the cells into a site of delayed hypersensitivity reaction in the former case to be compared with 'antigenic help' in the latter. The effect of Con A coupling alone was also assessed.

From the results of the experiments as shown in Table 1 and Fig. 1 the following effects can be clearly seen: (1) powerful augmentation of immunity is seen in the test animals (group h) which are immunised so as to produce specific tuberculin help in immunisation (Table 1 and Fig. 1). It is of interest that the specificity of the rejection assays with regard to 6a and 6b is well maintained (Fig. 1); (2) the BCG status of the animal itself does not confer any

Table 1 Immunisation groups using Con A-PPD

Immunisation regime	Antigenic Ratio	
	Control (no BCG given)	BCG-primed
Irradiated cells only	a 1.7	b 1.5
Irradiated cells reacted with Con A	c 1.6	d 1.5
Irradiated cells mixed with PPD	e 1.4	f 1.0
Irradiated cells coupled to PPD	g 1.2	h 10.7

12–20-week-old C57B1/10 ScSn (B10) mice (OLAC, Bicester) were immunised with one tenth of the human intradermal dose of BCG (Glaxo, Greenford) in 0.2 ml saline by intraperitoneal injection. After two weeks, groups of three BCG-primed and untreated mice were injected with the following subcutaneously into the right groin in 0.2 ml saline: groups a and b: 10^7 irradiated 6A cells, groups c and d: 10^7 6A cells reacted with Con A, groups e and f: 10^7 6A cells mixed with PPD, groups g and h: 10^7 cells coupled to PPD through Con A as described. A week later, all groups received 10^7 irradiated cells subcutaneously into the right groin as a booster immunisation. Ten days after this the mice were challenged with 2×10^5 live 6A cells injected subcutaneously in 0.1 saline also into the right groin. A group of immunised mice was also challenged. Tumour diameters were measured with calipers in two perpendicular planes and the mean taken. Results are expressed as an antigenic ratio: mean tumour diameter in unimmunised mice/mean tumour diameter in immunised mice. An antigenic ratio (A.R.) of greater than 1.0 indicates some immunity. The antigenic ratios shown are based on measurements on the sixteenth day after challenge.

increased immunity to the tumours (Table 1). If anything, the BCG-positive animals support tumour growth slightly better than do BCG-negative animals when uncoupled cells are used. In view of the widespread use of repeated BCG treatment in the immunotherapy of human tumours, it is worth pointing out that such repeated administration of BCG particularly at the tumour site is aimed at activating the macrophages, not at inducing tuberculin sensitivity; (3) there is no benefit to be seen from immunising with irradiated tumour cells into the site of the tuberculin reaction (Table 1). This is a manoeuvre that has in the past been claimed to give adjuvanticity⁷, but in the present model, benefit is not apparent; (4) Con A itself, when coupled to cells, produces no enhanced response (Table 1).

The rejection assay is believed to represent predominantly a cell-mediated reaction and therefore is likely to represent cooperation between the two sets of T cells. This potentiation effect clearly requires the 'carrier' molecule (tuberculin) to be coupled to the tumour cell rather than mixed with it and given into a pre-sensitised host. The conditions are thus typical of the carrier-hapten situation found in T cell-B cell cooperation, the PPD being the carrier and the tumour specific antigen, the hapten.

The tumours studied were selected for immunogenicity. Immunisation with three or more injections of irradiated tumour cells on their own is capable of producing a good degree of immunity. Further studies are in progress to see to what extent resistance can be achieved to tumours of lower degrees of immunogenicity.

The technique here described is of wide applicability, since Con A reacts well with nearly all cells and particularly with tumour cells, and the single Con A-PPD reagent can therefore be used for coupling PPD to different cells at will. Furthermore, since BCG has been frequently given without ill effect to humans with tumours, there would seem to be no ethical problem in extending studies of this kind to immunotherapy of human tumours.

K.S. holds an MRC Training Fellowship.

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Host-donor cellular interactions in the treatment of experimental osteopetrosis

OSTEOPETROSIS is a rare bone disease of man, animals and birds¹. A striking feature is the failure of resorption of bone during growth and a reshaping of the skeleton so that the medullary cavities of shaft bones, normally containing only soft tissue—bone marrow—are filled with unabsorbed primary spongiosa causing a characteristic opaque radiological appearance that gives the disease its name. The osteoclast, a multinucleated giant cell, is considered to be responsible for the resorption of bone², and it has long been held that osteopetrosis is due to deficient osteoclasts. That the defect had a cellular rather than a humoral basis¹ was indicated when it was shown

that bony resorption in osteopetrotic mice was resumed after short periods in parabiosis with normal litter mates^{3,4} (which also showed that a circulating cell was involved) and after infusions of phenotypically normal and histocompatible haematopoietic cells^{5,6}. The subsequent and often rapid disappearance of the calcified material suggested to us that a new and proliferating population of cells was responsible, and to test this donor cells were tracked by a chromosome marker technique⁷. We now report that only low proportions of donor cells were located.

We used microphthalmic mice which suffer from osteopetrosis and were derived from Grüneberg⁸ (G) and bred at the MRC Radiobiology Unit, Harwell. The recessive *mi* gene in the homozygous state determines osteopetrosis. The *mi* gene was bred into the standard CBA/H inbred strain^{4,6} and thus in various combinations with the G strain indicated in Tables 1–3 it was possible to use the T6 chromosome marker because CBA/H and CBA T6T6 inbred strains are immunologically uniform. Because of closed colony conditions the G strain has greatly reduced heterozygosity⁴ also allowing intra-strain transplantation.



Fig. 1 Radiographs of osteopetrotic tibias ($\times 8$). Left, 28-d-old untreated G *mi* mouse. Note absence of medullary cavity. Centre, G *mi* mouse injected intraperitoneally at birth with haematopoietic cells. At 175 d partial resorption (\pm) had occurred but the metaphysis and submetaphyseal shaft were osteopetrotic. Right, G *mi* mouse injected intravenously at 19 d with haematopoietic cells and killed 29 d later. Resorption was subtotal (+), only the metaphysis was osteopetrotic.

In the three experiments reported here the donated hybrid cells (T6T6 \times G)_{F1} were unable to react immunologically against the host—except by virtue of the H-Y antigen to which the stocks are poor responders. On the other hand, in some instances the host could formally react against non-H-2 antigens of the donated cells.

Resorption in shaft bones was evaluated at the times shown in Tables 1–3, and estimated arbitrarily by radiology and histology as 'partial' (\pm) when resorption had clearly started but which was far from complete, and 'sub-total' (+) when it was approaching completion. In our hands, a patch of osteopetrosis usually persisted at the growth plate of long bones (Fig. 1). The cytogenetic status was also estimated at the times shown in Tables 1–3. The behaviour of vertebrae, ribs and membrane bones are not described here. Serious defects such as blindness and failure of eruption of teeth were unaffected.

The results of the three experiments were as follows: (1) Microphthalmic G mice injected intraperitoneally at birth with bone marrow from normal hybrid (T6T6 \times G)_{F1} mice showed subtotal or partial resolution of the osteopetrosis within 2 months. Only low proportions of T6-marked donor cells were detected in the spleen and bone marrow (Table 1). Persistence of donor cells, formally histoincompatible, may be attributed to immunological tolerance of the newborn to non-H-2 antigens. (2) In older mice injected intravenously, the resolution of the osteopetrosis was partial and in none were donated cells detected a month and more after treatment (Table 2).

Table 1 Chimaerism and results of treatment of newborn microphthalmic mice by intraperitoneal injection of allogeneic bone marrow suspension from phenotypically normal T6-marked mice

mi no.	Host stock	Host age and sex	Donor stock and sex	Cell dose ($\times 10^{-6}$)	% Dividing donor stock cells in host		Degree of resolution of osteopetrosis at time (d)
80	G	< 24 h ♀	(T6T6 \times G)F ₁ ♀	5	Spleen	1	+ at 55
					Bone marrow	0	
81	G	< 24 h ♂	(T6T6 \times G)F ₁ ♀	5	Spleen	2	+ at 56
					Bone marrow	1.5	
82	G	1 d ♂	(T6T6 \times G)F ₁ ♀	7	Spleen	2	\pm at 58
					Bone marrow	0	
83	G	1 d ♀	(T6T6 \times G)F ₁ ♀	7	Spleen	2	+ at 58
					Bone marrow	1	

Microphthalmic mice are difficult to maintain; as they are edentulous, they are weaned onto a wet mash. The T6-marked cells were detected by a standard cold spread method⁷ and 100–300 metaphase plates were scored in all preparations. The T6 marker detects dividing cells but does not reveal their identity.

Table 2 Results of treatment of weanling microphthalmic G mice by intravenous injection of allogeneic bone marrow suspension from phenotypically normal T6-marked mice

mi no.	Host stock	Host age and sex (d)	Donor stock and sex	Cell dose ($\times 10^{-6}$)	% Dividing donor stock cells in host		Degree of resolution of osteopetrosis at time (d)
181	G	♀ 18	(T6T6 \times G)F ₁	5	Spleen	0	— at 35
					Bone marrow	—	
182	G	♀ 18	(T6T6 \times G)F ₁	5	Spleen	0	\pm at 62
					Bone marrow	—	
183	G	♂ 18	(T6T6 \times G)F ₁	5	Spleen	0	\pm at 90
					Bone marrow	0	
184	G	♀ 22	(T6T6 \times G)F ₁	47	Spleen	—	\pm at 117
					Bone marrow	0	
185	G	♂ 22	(T6T6 \times G)F ₁	47	Spleen	0	\pm at 117
					Bone marrow	0	
189	G	♀ 21	(T6T6 \times G)F ₁	47	Spleen	0	\pm at 46
					Bone marrow	0	
190	G	♀ 20	(T6T6 \times G)F ₁	47	Spleen	0	\pm at 46
					Bone marrow	0	
191	G	♂ 19	(T6T6 \times G)F ₁	47	Spleen	0	\pm at 29
					Bone marrow	0	

Table 3 Chimaerism and resolution by parabiosis of mature microphthalmic mice with normal (T6T6 \times G)F₁ hybrids

mi no.	Osteopetrotic stock and sex		Normal stock partner and sex	Days in parabiosis	% T6-marked dividing cells in petrotic mice		Degree of resolution of osteopetrosis at time (d)
35	G	♂	(T6T6 \times G)F ₁ ♂	14	Spleen	no result	\pm at 120
					Bone marrow	0	
153	(G \times CBA)F ₁	♂	(T6T6 \times G)F ₁ ♂	13	Spleen	32	\pm at 120
					Bone marrow	3	
155	(G \times CBA)F ₁	♂	(T6T6 \times G)F ₁ ♂	13	Spleen	8	+ at 120
					Bone marrow	1	
					Thymus	3	
					Blood	32	
156	(G \times CBA)F ₁	♂	(T6T6 \times G)F ₁ ♂	13	Spleen	15	\pm at 120
					Bone marrow	0	
					Thymus	5	
					Blood	29	

Mice were anaesthetised with avertin and parabiosed by the coelomic method. Braided silk (5/0) was used to suture the abdominal walls and the skin incision was closed with metal clips. At separation the normal anatomy was restored. Pairs were housed individually and received food pellets, wet mash and tap water *ad libitum*. To increase the number of metaphases for scoring, this group received sheep red blood corpuscles intraperitoneally 4 d before being killed. Previous experiments had shown that while the bone marrow was mitotically active the spleens were not. Separated leukocytes from the peripheral blood were cultured by Festenstein's method⁸.

(3) Four microphthalmic mice, after parabiosis to normal (T6T6×G)F₁ hybrids, showed partial resolution of osteopetrosis. Four months later, cells were detected in the haematopoietic tissues in the three syngeneic matchings, the greater proportion in the spleens and peripheral blood no doubt being due to antigenic stimulation (Table 3).

The results indicate that in a syngeneic combination (3) or when immunological tolerance of the newborn can be invoked in allogeneic matches (1), donated cells may persist in the microphthalmic recipient but in very small proportions. In the allogeneic match (2) no such cells were found 1 month later (compare the loss of grafts after 1 month in similar skin grafting experiments⁴). Nevertheless all groups showed resolution of the osteopetrosis to a greater or lesser degree in individuals.

It is possible that the weanlings in group (2) and the one successful case of parabiosis across an immunological barrier, where no donated cells were subsequently found, did have small and undetected populations of donor cells, which further experiments with mitotic stimulants may reveal. For the present, however, we postulate that allogeneic donor cells were eliminated but during their limited period of residence had caused considerable resorption of bone by providing osteoclastic precursors or had triggered the recipient's osteoclasts into effective function, comparable to the T-B cell-cell interactions of immunology. Experiments are in progress to investigate whether the resolution of osteopetrosis is permanent in these conditions, which would support the 'trigger' theory.

In experiment (1) and even in (3) where the spleen and separated leukocytes from the peripheral blood were under stimulation by foreign antigen, the donor population was in a minority and there was no 'take over', as would be presumed to have occurred in Walker's⁵ experiments where the recipients had been lethally irradiated before treatment.

We thank Mr M. J. Haddaway, Mr M. J. Corp and Mr P. Adams for radiology and Mr C. Williams for histology.

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Cellular localisation of human urogastrone/epidermal growth factor

HUMAN urogastrone (hUG) is a polypeptide prepared from urine and is chemically similar to mouse epidermal growth factor (mEGF) prepared from submandibular salivary glands. Both these substances stimulate fibroblast proliferation in tissue culture systems. hUG and mEGF inhibit gastric acid secretion in experimental animals and hUG produces an identical effect in man. We show here, using immunofluorescent techniques,

that hUG is present in the duct cells of human submandibular gland and in Brunner's gland cells in the first part of the duodenum. This work suggests that these polypeptides may have a role in mucosal growth and control of gastrointestinal secretion.

The fact that peptic ulcer undergoes remission in pregnancy¹ led to the discovery of a powerful inhibitor of gastric acid secretion termed urogastrone in urine. This inhibitor has been shown to consist of a single polypeptide chain of 53 amino acids containing three disulphide bonds². Independently, Cohen and his colleagues³ have isolated a substance from mouse submaxillary glands known as epidermal growth factor (mEGF),

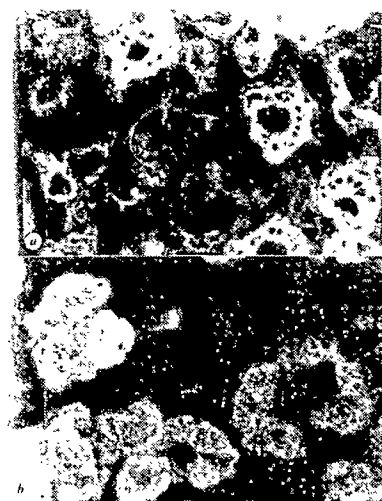


Fig. 1 Fluorescence can be seen as the light areas in *a*, the cells of the ducts of the submandibular glands and *b*, in the cells of the glands of Brunner in the first part of the duodenum.

which is closely related structurally to urogastrone and in biological activity it seems to be identical⁴. Both are powerful mitogens causing epithelial proliferation and keratinisation of squamous epithelial cells. They both inhibit gastric acid secretion and furthermore, they share common receptors in cultured human fibroblasts⁵. Preliminary physical data on a human epidermal growth factor (hEGF)⁶ suggests that the material in question is urogastrone².

Purified human urogastrone is an effective inhibitor of gastric acid secretion in normal male subjects⁷ and in patients with duodenal ulcer⁸ and Zollinger-Ellison syndrome, a disease due to an abnormally high circulating level of the hormone gastrin⁹. The cells of origin, storage or secretion of urogastrone, however, have not been identified in man. Urogastrone can be detected in human urine after a variety of surgical ablative procedures¹⁰, whereas (mEGF) has been found in specialised cells of the submandibular glands¹¹, and this finding prompted the present enquiry.

Antibodies to hUG were raised in rabbits¹². Specimens of the following normal human tissues were placed in polythene bags immediately after surgical excision, fixed in liquid nitrogen at -70 °C for 10 min, and were subsequently stored at -25 °C for further processing: submandibular salivary glands, parotid salivary glands, oesophagus, antrum and fundus of stomach, duodenum, jejunum, colon, breast, pancreas, ovary, adrenal, thyroid, kidney, bladder, liver, prostate, smooth muscle, skeletal muscle, cardiac muscle and thymus were studied. Frozen sections were cut and the slides exposed for 20 min to specific rabbit hUG antiserum without dilution and at 1 : 20 dilution using saline. The slides were washed for 20 min, and sheep anti-rabbit fluorescein-labelled immunoglobulin (Wellcome) was applied for a further 20 min followed by a final rinse. The

sections were then examined under ultraviolet light. Control sections from the same tissues were processed using nonspecific rabbit serum and also in a separate series using hUG absorbed specific rabbit antiserum. Adjacent sections of all tissues were stained with conventional haematoxylin and eosin to allow identification of cell types more easily in comparison with the fluorescein-labelled sections. An alternative method of fixation using buffered picric acid formaldehyde (pH 7.3) for 48 h before section cutting and application of the double layer fluorescence technique gave similar results to those obtained with the cryostat material. Fluorescence was seen in the cells of the ducts of human submandibular salivary glands and in the cells of Brunner's glands of the duodenum (Fig. 1a, b). Examination of sections from all the other tissues and of all control sections failed to reveal any appreciable fluorescence. An alternative freeze-drying and formaldehyde vapour fixation technique followed by immunostaining procedures has been reported¹⁸ to give an extremely clear picture of localisation of mEGF, but is much more time consuming than the methods outlined here for human tissues.

Our tests indicate that immunoreactive urogastrone/epidermal growth factor is present in specialised cells of the human submandibular salivary gland and also in the cells of the glands of Brunner in the duodenum. These findings may explain why in previous studies significant amounts of urogastrone could be detected in urine following different surgical ablation procedures in man¹⁰. No case examined so far has involved ablation of these two specific areas.

Our results also raise the question of the nature of the gastric acid inhibitory hormone 'enterogastrone', said to be liberated from the duodenum by fat ingestion in the studies of Kosaka and Lim¹⁴ (reviewed in detail by Gregory¹⁵). Could this 'enterogastrone' be urogastrone, a well-established potent inhibitor of gastric acid secretion and now localised in Brunner's glands? Furthermore, it is difficult to avoid speculation that the presence of a powerful growth factor at two sites in the gastrointestinal system is probably related to the known rapid turnover of the cells lining the gut¹⁶.

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Renal actions of prostacyclin

A NEW and important member of the arachidonate-thromboxane-prostaglandin system, prostacyclin (PGI₂), has recently been isolated¹⁻³. This bicyclic prostaglandin has been shown to be synthesised from the prostaglandin endoperoxides PGG₂ and PGH₂, by arteries, veins, heart and other tissues in several species, including man¹⁻⁷. PGI₂ is a powerful inhibitor of platelet aggregation and relaxes arterial strips^{1,2,4,5}. It is 30 times more potent than PGE₁ as an inhibitor of platelet aggregation, and in the intact dog promotes a dramatic reduction in systemic blood pressure^{1,2,8,9}. It has also been suggested that PGI₂ is a potent coronary vasodilator and anti-atherogenic compound¹⁰⁻¹², and that it plays an essential part in the homeostasis of the cardiovascular system^{1,2,4,5,11,12}. The kidneys receive a large portion of the cardiac output and have an extensive vascular tree, and so PGI₂ may be extremely significant in regulating kidney function. Human and rabbit renal cortical microsomes have been found to convert PGG₂ to PGI₂ (ref. 13), but nothing is known of the renal effects of PGI₂. Therefore, this study was designed to evaluate the effects and significance of an intrarenal infusion of PGI₂ on renal haemodynamics, urinary function and renal vein plasma renin activity, and to compare these effects with those produced by PGE₂ and PGD₂, two prostaglandins known to be synthesised by the kidney and to affect renal function.

Table 1 shows the effects of intrarenal infusions of PGI₂, PGD₂ and PGE₂. All three prostaglandins markedly elevated renal blood flow. The increases in blood flow produced by PGI₂ and PGD₂ were due to an enhanced blood flow to both the outer and inner cortical regions as shown in Table 1. The increases in blood flow to the inner cortex were greater than those in blood flow to the outer cortex, thus shifting the intrarenal distribution of blood flow to the inner cortex. Although no values for the PGE₂ experiments are shown, radionuclide-labelled microspheres were used in one experiment, and results similar to those seen with PGI₂ and PGD₂ were obtained.

All three prostaglandins produced similar increases in renal blood flow without a change in glomerular filtration rate. Their effects on urinary function, however, were dissimilar (Table 1), both PGI₂ and PGE₂ promoting a natriuresis and diuresis, and PGD₂ having no significant effect on the urinary flow rate and the fractional excretion of sodium. In addition, PGI₂ and PGE₂ but not PGD₂, significantly elevated the excretion rate of potassium (not shown). At no time during the infusion of these prostaglandins were changes noted in systemic blood pressure, heart rate or contralateral renal function, indicating that at the dosage employed, the effects induced by these prostaglandins were restricted to the infused kidney. In two experiments, the PGI₂ solvent and the PGI₂ metabolite, 6-keto-PGF_{1α}, were infused separately and found to have no significant effect on renal blood flow, urinary output or renal vein plasma renin activity. This implies that neither the solvent nor the metabolite were responsible for the renal effects induced by PGI₂.

In a previous report, we demonstrated that the infusions of PGD₂ and PGE₂ at a 10-fold higher rate of 0.4 µg per kg of body weight per min produced changes in renal function qualitatively similar to the effects elicited by PGD₂ and PGE₂ in this study¹⁴. No systemic effects were noted. PGI₂ at this higher rate of infusion (0.4 µg per kg per min) decreased systemic blood pressure and increased heart rate. This indicates that PGI₂ leaving the kidney was not effectively metabolised by the lungs, and is consistent with the observation made earlier by Waldman *et al.*¹⁵ that the vasodepressor response of PGI₂ in the dog is not diminished by passage through the lungs, in contrast to a dramatic reduction in the vasodepressor effect of PGE₂. This phenomenon might be explained in one of three ways. First, PGI₂ undergoes little metabolism in the lung;

second, PGI₂ undergoes some metabolism in the lung, but because of its marked potency, enough escapes to cause systemic effects; third, the amount of PGI₂ reaching the lung during the higher infusion rate exceeded the capacity of the lung to metabolise it. These observations suggest that if PGI₂ is formed and released by the kidney in sufficient quantity, it might function as a circulating vasodepressor substance.

The pattern of blood flow changes induced by the three prostaglandins is similar to that produced by the prostaglandin precursor, arachidonic acid, and other renal vasodilators^{16,17}. Their similar haemodynamic effects, but differing effects on urinary function suggest that PGI₂ and PGE₂, but not PGD₂, either directly and/or indirectly inhibit sodium and water reabsorption. Although the present experiments do not elucidate the mechanism by which PGI₂ promotes a natriuresis and diuresis, PGE₂ has been shown to inhibit directly the reabsorption of sodium in the nephron^{18,19}.

Of the three prostaglandins, only PGD₂ significantly elevated renal vein plasma renin activity when infused at a rate of 0.04 µg per kg per min. Vander²⁰ also observed that PGE₂ infusion in a similar dose did not alter renin release. In a previous series of experiments, however, we found that when PGE₂ was infused at a 10-fold higher rate (0.4 µg per kg per min) renal vein plasma renin activity was increased¹⁴; Yun *et al.*²¹ also found this. A comparison of the haemodynamic and urinary effects of PGE₂ at the two doses (0.04 and 0.4 µg per kg per min) used in our studies revealed that the higher dose produced an increase in renal blood flow which was twice as great as that produced by the lower dose, while the diuretic and natriuretic effects were not appreciably different at the two dose levels. Previous demonstrations of increased renin production following administration of arachidonic acid or PGE₁ led to the suggestion that prostaglandins may act directly on the juxtaglomerular cells to release renin. This conclusion

has been questioned, as it was pointed out that the accompanying natriuresis *per se* may have triggered renin release by presenting more sodium to the macula densa²². The present observation might be explained by precisely the opposite argument, that the renal haemodynamic effects induced by PGI₂ and PGE₂ are promoting renin release whereas the natriuresis is inhibiting release. Our additional experiments have used a non-filtering kidney model in which the delivery of sodium to the macula densa is prevented; the infusion of either PGI₂ or PGE₂ at the lower dose (0.04 µg per kg per min) then increased renin release. In the intact kidney, either an elevated sodium load at the macula densa as suggested by Vander²³, or an action of PGE₂ and PGI₂ to reduce ion flux across the macula densa as postulated by Thureau *et al.*²⁴ may have blunted an increase of renin release. The stimulation of renin release induced by PGD₂ is unopposed because of the absence of a natriuresis.

Our evaluation of PGI₂ indicates that when infused intrarenally it produces changes in renal haemodynamics and urinary function which are qualitatively identical to those produced by PGE₂. It is impossible at present to state which prostaglandin (PGI₂, PGE₂ or PGD₂) is the major renal prostaglandin mediating the effects of endogenous arachidonate released following neural and hormonal stimulation. This will be resolved by the future development of analytical techniques which will permit measurement of the levels of renal prostaglandins in physiological conditions. However, it is noteworthy that PGI₂ escapes metabolism by the lung; if the kidney is a major source of PGI₂ it may have significant effects beyond that organ. Conversely, if arterial levels of PGI₂ are sufficiently high, production of PGI₂ elsewhere in the body may have significant effects on renal function. It should be noted, however, that PGI₂ did not exhibit any superior potency to PGE₂ in its effects on renal function. Finally, we obtained

Table 1 The effects of intrarenal infusions of PGI₂, PGD₂ and PGE₂

Infusion periods	RBF (ml per min per g of kidney)	OCBF (ml per min per g of tissue)	ICBF (ml per min per g of tissue)	RF	V̇ (µl per min per g of kidney)	FE _{Na} ⁺ (%)	RVPRA ng per ml per h
	N = 9	N = 8	N = 8	N = 8	N = 9	N = 9	N = 5
Control	4.1 ± 0.6	8.0 ± 1.2	4.4 ± 0.6	1.9 ± 0.1	7 ± 1	0.94 ± 0.25	5.6 ± 1.5
PGI ₂	6.1 ± 0.7*	10.7 ± 0.8*	9.7 ± 0.8*	1.2 ± 0.1*	15 ± 2*	2.03 ± 0.56*	7.0 ± 2.2
	N = 8	N = 6	N = 6	N = 6	N = 8	N = 8	N = 6
Control	4.2 ± 0.6	7.9 ± 1.1	4.4 ± 0.8	2.0 ± 0.2	20 ± 4	1.40 ± 0.46	3.8 ± 1.3
PGD ₂	7.3 ± 1.2*	14.9 ± 2.7*	13.3 ± 2.7*	1.2 ± 0.1*	25 ± 4	1.75 ± 0.50	8.8 ± 2.2*
	N = 7				N = 7	N = 7	N = 6
Control	4.4 ± 0.5	—	—	—	12 ± 3	1.58 ± 0.52	3.9 ± 1.5
PGE ₂	6.2 ± 0.7*	—	—	—	38 ± 10*	3.54 ± 0.88*	4.6 ± 1.6

Values (right renal) are expressed as the mean ± s.e.m.; N, no. of animals; *, *P* < 0.05 as determined by the paired *t* test. RBF, renal blood flow; OCBF, outer cortical blood flow; ICBF, inner cortical blood flow; RF, ratio of cortical blood flows (outer cortical to inner cortical blood flows); V̇, urinary flow rate; FE_{Na}⁺, fractional excretion of sodium; RVPRA, renal vein plasma renin activity. OCBF, ICBF, RF and RVPRA were not determined in all experiments because of technical difficulties. OCBF, ICBF and RF mean values are not shown for PGE₂ experiments, because intrarenal blood flow was not determined. Mongrel dogs of either sex (N = 22) were fasted 18 h before the experiment and allowed free access to tap water. The animals were anaesthetised with intravenous pentobarbital (30 mg per kg of body weight) and placed on a positive pressure respirator (Harvard Apparatus). Indwelling polyethylene catheters were inserted into the following vessels; the right femoral artery for monitoring of systemic blood pressure and collection of blood samples, the left femoral artery for advancement into the left ventricle, the left femoral vein for advancement into the right renal vein and the right femoral vein for the infusion of inulin to measure the glomerular filtration rate. A suprapubic midline abdominal incision was made and both ureters were cannulated. A right retroperitoneal flank incision was performed, and a hydraulic cuff (*In Vivo* Metric Systems) and electromagnetic flowprobe (Biotronix Laboratories) were placed on the renal artery. A 25-gauge infusion needle was inserted into the artery proximal to the flowprobe and hydraulic cuff, and normal saline was infused at 0.5 ml min⁻¹. One hour was allowed for stabilisation of renal function. Each experiment comprised two 15-min infusion periods (control saline infusion followed by PGI₂, PGD₂ or PGE₂ (0.04 µg kg⁻¹ min⁻¹) infusion), with three 5-min urine collections in each period. After the start of the second urine collection, arterial samples for inulin, sodium and potassium and renal vein samples for renin were collected. Midway through each period radionuclide-labelled microspheres (15 ± 5 µm ⁸⁵Sr, ¹⁴¹Ce and ⁵¹Cr, New England Nuclear) were introduced into the left ventricle with the exception of the PGE₂ experiments. PGD₂ and PGE₂ were dissolved in ethanol and stored at -20 °C until the day of the experiment, when the ethanol was evaporated off and the prostaglandins were dissolved in normal saline. PGI₂ was chemically synthesised according to the method of Corey *et al.*²⁵. It was dissolved in water and stored at -70 °C until several hours before the experiment; an aliquot of the stock solution was then dissolved in a Tris buffer at pH 9. At the end of this prostaglandin infusion period, the animals were killed and the kidneys removed, weighed and sectioned according to the method of Slotkoff *et al.*²⁶ for counting of radioactivity. Renin levels were measured by radioimmunoassay.

data which suggests that the net effect of either PGI_2 or PGE_2 on renin release is the summation of its stimulatory action by means of haemodynamic changes and/or a direct stimulation of the juxtaglomerular apparatus, versus a dampening effect produced by altered sodium transport at the macula densa.

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Temperature-induced phase shift of daily rhythm of serum prolactin in gulf killifish

DAILY variations in circulating levels of the pituitary hormone prolactin have been reported in several vertebrates, including fishes¹⁻⁴. In some animals, the 24-h rhythm changes seasonally with respect to the time of day that maximum and minimum prolactin levels occur^{4,5}. It has been hypothesised that this seasonal change in phase of prolactin rhythm is an important component of the mechanism controlling seasonality in vertebrates⁶. Because water temperature is generally considered the principal environmental regulator of seasonal changes in reproduction and metabolism in many fishes, including the gulf killifish *Fundulus grandis*⁷, we determined the daily rhythm of serum prolactin concentrations in fish held at temperatures that are stimulatory (20 °C) or inhibitory (28 °C) for reproductive development. We found that an increase in water temperature from 20° to 28 °C phase shifts the daily variation of serum prolactin with respect to the daily photoperiod in *F. grandis*.

There were variations in serum prolactin concentration in gulf killifish maintained at water temperatures of either 20° or 28 °C ($P < 0.01$; $P < 0.05$, one-way analysis of variance). However, the phase relationships of the peaks and troughs of the two rhythms differed with respect to the daily photoperiod 12 h light and 12 h dark (12L:12D). Thus, the peak

level of prolactin in fish acclimated to 20 °C occurred at 1400 h (8 h after the onset of light) whereas the prolactin peak in fish acclimated to 28 °C occurred at 0600 h (onset of light) (Fig. 1). There was a highly significant interaction between the temperature of acclimation and the time of day of sampling ($P < 0.01$,

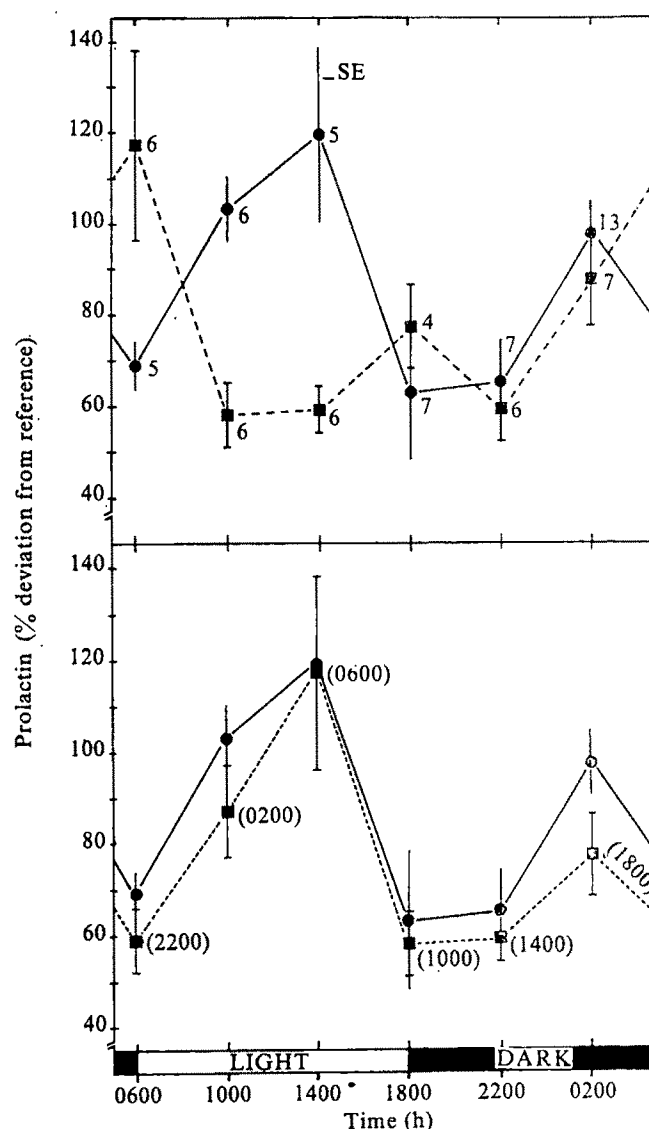


Fig. 1 Serum prolactin levels of gulf killifish acclimated to either 20 °C (●) or 28 °C (■). Both groups of fish were kept in a 12L:12D photoperiod regime—with light onset at 0600 h. The upper graph depicts the two unadjusted rhythms; sample size is adjacent to the mean prolactin levels, vertical bars represent standard error of the mean. In the lower graph the points depicting the prolactin rhythm of the 28 °C acclimated fish have been adjusted by 8 h to demonstrate the similarity in form of the two rhythms. Actual sampling times of the 28 °C acclimated fish are shown in parentheses. Fish of both sexes were collected from Grand Isle, Louisiana in February and maintained indoors in 70-l aquaria which received filtered and aerated water (salinity 3‰) and approximately 2 g of flake food (TetraMin) once daily. Routine maintenance and feeding were done at random times during the day. After a 2-week preliminary acclimation at 20 °C and 12L:12D (approximately the March photoperiod regime of Grand Isle) fish were divided into two groups which received either 20° or 28 °C, 12L:12D and 3‰ salinity were maintained for both groups. After an additional 2 weeks of acclimation, the fish were killed at one of six different times of day. Blood was taken by heart puncture, allowed to clot under refrigeration and centrifuged. The serum was assayed with a double antibody technique utilising an antiserum to pollock prolactin and iodinated ovine prolactin¹². Because killifish prolactin was not available, we were unable to determine absolute levels of prolactin. However, a pooled reference standard was run which allowed us to ascertain relative differences in circulating titres of prolactin. Mean levels of prolactin were not significantly different between the sexes.

two-way analysis of variance). This interaction is due to differences in the phase relationship of the two rhythms. The significant interaction can be eliminated by shifting the rhythm of the fish acclimated to 28 °C to 8 h later in the day (Fig. 1). The mean serum prolactin concentrations (mean of all values throughout the day) did not differ between the two groups.

Our work reconfirms the often cited warning to researchers of the problems inherent in sampling at one time of day with the added caution that environmental factors other than photoperiod (for example, temperature) may influence hormone rhythms considerably. Further, the phase shift in the prolactin rhythm we induced by a temperature change may have important implications in the understanding of the seasonal physiology of the gulf killifish. Increased water temperature causes gonadal regression in several species of fish⁸ including *F. grandis*. Gulf killifish collected from their natural environment have ripe gonads when ambient temperatures are 20 °C (early March) and regressing gonads when ambient temperatures reach 28 °C (May–June) and higher (July–August)^{7,9,10}. In our experiment male killifish acclimated to 28 °C had a significantly lower gonadosomatic index ((gonad weight/body weight) × 100) than those acclimated to 20 °C ($P < 0.05$ Student's *t* test). Apparently, the effects of temperature on the gonads may be mediated by altering the phase of the prolactin rhythm. In male killifish maintained on a 15L:9D regimen, daily injections of prolactin 8 h after the onset of light (the time of peak prolactin levels in the fish acclimated to 20 °C) stimulated the reproductive system whereas prolactin injections at other times of day including light onset (the time of peak prolactin in fish acclimated to 28 °C) were ineffective⁷.

The annual cycle of reproduction in teleost fishes is regulated by changes in daylength, temperature or a combination of both⁸. The strategy used seems to depend more on ecological demands than on phylogenetic relatedness. As in birds and mammals⁸, circadian mechanisms control reproduction in a photosensitive teleost¹¹. Our results indicate that temperature control of reproduction in an ectothermic thermosensitive species may also be mediated in part by circadian systems.

We thank Albert J. Fivizzani for reviewing the manuscript. This research was supported in part by a grant from the NSF (PCM76-06841) to R.E.S. and A.H.M.

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Cyclic AMP and noradrenaline sensitivity fluctuations in regenerating newt tissue

Limb regeneration in the adult newt is dependent on the nervous system^{1,2} but the biochemical events involved are not well understood. A role for catecholamines (CA) seems likely since histofluorescence has shown that CA are more abundant in regenerates than in other newt limb tissues³. It has also been shown that reserpine, which promotes the release of CA from nerve terminals, accelerates regeneration⁴, whereas chlorpromazine⁵, guanethidine⁵ (both CA release blockers) and α -methyl-*p*-tyrosine⁶ (CA biosynthesis inhibitor) inhibit it. Studies suggest that in many biological systems the CA effects are linked to the activation of membrane adenylate cyclase with subsequent production of cyclic AMP⁷. It has also been suggested that nerves may transmit trophic information by the stimulation of this enzyme^{8–11}. To test this hypothesis further, regenerates were isolated either for direct measurements of cyclic AMP or for stimulation with CA *in vitro*¹². This method avoids the possible nonspecific effects due to systemic administration of CA. We now report that the fluctuations of cyclic AMP found at the various stages of regeneration correlate with noradrenaline (NA) sensitivity changes.

Adult newts *Triturus cristatus* were housed and handled and their limbs amputated as before⁶. Characterisation of the stages was performed on a morphological basis¹³, which is the most reliable criterion for specimen selection¹⁴. In order to measure true endogenous levels of cyclic AMP, isolated regenerates were collected in 0.25 ml of batracian Ringer¹⁵ and immediately heated in a boiling water bath for 10 min. A pool of 3–10 regenerates, depending upon the amount of regenerating tissue, was routinely used. The cyclic AMP accumulation induced by noradrenaline (or

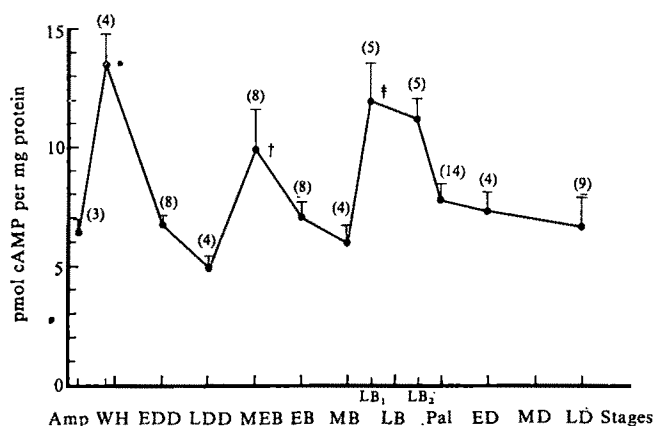


Fig. 1 Endogenous cyclic AMP levels (pmol per mg protein) in newt forelimb regenerates at various stages of development. The various stages were designed as described elsewhere¹³. Amp = amputation, WH = wound healing, EDD = early dedifferentiation, LDD = late dedifferentiation, MEB = moderate early bud, EB = early bud, MB = medium bud, LB₁ = early LB, LB₂ = late bud, LB₂ = late LB, PAL = palette, ED = early digits, MD = medium digits, LD = late digits. Regenerating tissue was removed with iridectomy scissors from live animals placed under binocular magnifying glass. After removal, the tissue was immediately plunged into a glass homogeniser (containing 0.25 ml of Ringer solution) kept in boiling water. The heat inactivation was continued for 10 min. A pool of 10 (WH) to 3 (LD) regenerates was used for 1 homogenisation, the total number of experimental tubes at various stages being given in parentheses (= *N*). The tissues were then homogenised, at 4 °C, and centrifugation (4,000 r.p.m.) was performed at the same temperature. The pellets were used for protein determination²². Duplicate samples (50 μ l) of the supernatant were taken for the measurement of cyclic AMP using a saturation assay method²³. Values are mean \pm s.e.m. Statistical analysis was performed using Student *t* test for unpaired data. *, Significantly different from previous ($P < 0.01$) and next ($P < 0.001$) stages. †, Significantly different from LDD ($P < 0.05$). ‡, Significantly different from MB and Pal Stages ($P < 0.05$).

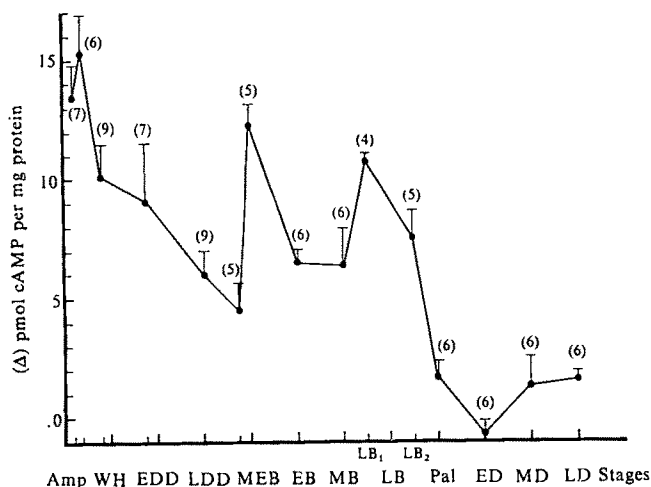


Fig. 2 Cyclic AMP increase (pmol per mg protein) induced by 10^{-4} M noradrenaline in newt forelimb regenerates at various stages of development. Pools of isolated regenerates were pre-incubated for 20 min at 35°C in 0.25 ml of Ringer solution¹⁵. After addition of theophylline (final concentration 5×10^{-3} M), which prevents the hydrolysis of cyclic AMP by phosphodiesterase, an incubation was carried out at 35°C for 10 min (total volume 0.33 ml) with noradrenaline. Controls were incubated in the absence of noradrenaline. The tissues were then inactivated by heat (see Fig. 1 for other experimental details). The increase in cyclic AMP above control is expressed as Δ . Values are mean (Δ) + s.e.m. (vertical bars) for the number of samples shown in parentheses (= N). In the absence of noradrenaline, the cyclic AMP values (pmol per mg protein) were 1.9 (WH 24 h), 6.8 (WH 48 h), 1.9 (WH 4 d), 3.4 (WH-EDD), 2.6 (LDD), 3.7 (late LDD), 2.7 (MEB), 3.3 (EB), 3.7 (MB), 4.0 (LB), 3.2 (LB₂), 4.2 (Pal), 4.4 (ED), 4.0 (MD) and 3.6 (LD). The abbreviations of the various stages are given in Fig. 1.

other adrenergic agonists) was measured as before¹². Some experiments were performed in the presence of an α - or β -adrenergic antagonist. Figure 1 shows that endogenous cyclic AMP levels fluctuate as a function of the stage of regeneration. Three distinct peaks of cyclic AMP were found and these occurred at the wound-healing (WH), at the moderate early bud (MEB) and at the late bud (LB) stages. The lowest values of cyclic AMP were found at the late dedifferentiation stage (LDD). Three peaks of cyclic AMP accumulation were also observed when isolated regenerates were exposed to 10^{-4} M NA (Fig. 2). In some experiments, pieces of tissue located under the wound were also exposed to NA and the results compared to those obtained with regenerates of the same forelimbs (Fig. 3). Regenerating tissues reacted to NA by a large increase in cyclic AMP concentration, while stump tissues did not. This implies that NA sensitivity differences may

exist between growing and resting limb tissues. Strikingly, the peaks resulting from NA stimulation (Fig. 2) roughly correspond to the peaks of endogenous cyclic AMP levels (Fig. 1). Incubation of regenerates at the WH and MEB stages was also performed in the presence of isoproterenol (ISO) or dopamine (DA). The former catecholamine stimulated cyclic AMP accumulation, while DA had no effect (data not shown). Moreover, in the presence of the β -adrenergic receptor antagonist, propranolol, or phentolamine, only the former was able to inhibit the cyclic AMP increases induced by NA (Table 1) or ISO (not shown). These results suggest that trophic information mediated by CA involves β -adrenergic receptors.

Thus, during the process of regeneration, fluctuation occurs in endogenous cyclic AMP levels and sensitivity of the tissue receptors to NA. It is tempting to correlate nerve activity, cell sensitivity to NA, endogenous cyclic AMP and the passage of one stage of regeneration to another. The first peak of cyclic AMP could be associated with the wound healing process (possibly linked to cell movements¹⁶) and the drop noted at LDD could be necessary for the occurrence of cell dedifferentiation. The following peaks might then be related to bursts of proliferation¹⁷, possibly linked to one or several phases of the cell cycle and to differentiation^{18,19}. Very recently, it was found that cyclic GMP levels also change during newt limb regeneration²⁰

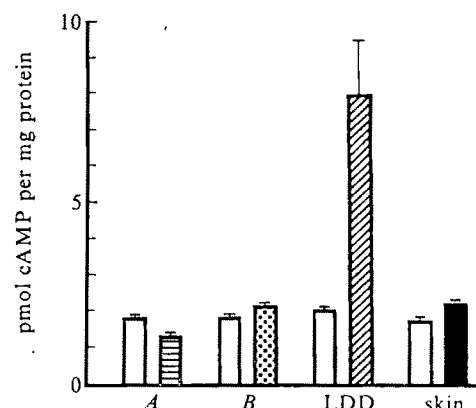


Fig. 3 The effects of 10^{-4} M noradrenaline on the cyclic AMP concentration (pmol per mg protein) in regenerating or non-regenerating tissues of the newt. Regenerates at the LDD stage were removed (see Fig. 1), as well as skin of nonregenerating tissues dissected from the region distant from the wound A, or adjacent to it B. Empty bars are controls, filled bars represent noradrenaline-treated tissues. The experimental details were given in Fig. 2. Values are mean + s.e.m.

and the low values of cyclic AMP observed at LDD (Fig. 1) seem to coincide with high levels of cyclic GMP at the same stage²⁰. Fluctuations of both cyclic nucleotides in a related but opposite way may then be needed during the process of regeneration, at least at dedifferentiation stages, and it is possible that the cyclic GMP effects are mediated by another nerve trophic factor of peptide nature²¹. Such speculations are currently under investigation using newt forelimb regenerates.

Partially supported by SNSF 3.544.075 (M.S.). We thank Dr Lawrence J. Fischer for help with the preparation of the manuscript, Miss C. Blank for technical assistance and Mrs N. Maalaoui for typing.

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Table 1 The effects of adrenergic receptor antagonists on the cyclic AMP accumulation induced by 10^{-4} M noradrenaline in newt regenerates of wound healing stages

WH-stage regenerates	pmol per mg protein
Control	6.0 ± 1.2 (4)
Noradrenaline	17.9 ± 2.8 (5)
Noradrenaline + phentolamine	15.8 ± 2.0 (3)*
Noradrenaline + propranolol	3.4 ± 0.3 (5)†

Control regenerates or regenerates exposed to noradrenaline were treated as described in Fig. 2. In experiments with an antagonist (final concentration 10^{-4} M), phentolamine or propranolol were added just before noradrenaline for the final 10 min incubation. Values are means \pm s.e.m. for the number of samples given in parentheses (= N).

*Not significantly different from noradrenaline-treated regenerates ($P > 0.6$, Student's t test).

†Not significantly different from control-regenerates ($P > 0.05$) and significantly different from noradrenaline-treated regenerates ($P < 0.001$).

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Increases in cyclic GMP levels may not mediate relaxant effects of sodium nitroprusside, verapamil and hydralazine in rat vas deferens

It has been suggested that increases in cyclic GMP levels may be responsible for the promotion of contractions in a variety of smooth muscles¹⁻⁶. This suggestion was based on observations that agents known to be capable of contracting smooth muscles produced marked increases in cyclic GMP levels in the respective muscles¹⁻⁸. However, recent reports have concluded that, although increases in cyclic GMP levels do occur during contractions in some types of smooth muscle, the increases in cyclic GMP levels are not responsible for the initiation or promotion of the contractions⁹⁻¹². Part of the evidence for this conclusion was the observation that marked increases in cyclic GMP levels occurred during relaxation of smooth muscles by drugs such as nitroglycerol^{10,12}. Schultz *et al.*¹³ have shown that other smooth muscle relaxants including sodium nitroprusside, hydralazine, and D-600 (a methoxy derivative of verapamil) are all capable of increasing cyclic GMP levels in isolated segments of rat vas deferens. These authors also noted that the 8-bromo derivative of cyclic GMP was itself capable of relaxing the rat vas deferens in certain conditions¹³. It was concluded that, not only was cyclic GMP not a mediator of smooth muscle contraction, but it might in fact be responsible for the smooth muscle relaxant effects of the above drugs. We describe here studies performed in rat vas deferens. Our results are not consistent with the hypothesis that increases in cyclic GMP levels are responsible for drug-induced smooth muscle relaxation.

Schultz *et al.*⁸ have suggested that the increases in cyclic GMP levels which accompany smooth muscle contractions might be part of a negative feedback mechanism tending to reduce calcium influx or to increase calcium efflux, thus limiting or terminating the contractions. The suggestion that cyclic GMP may mediate the relaxant effects of some drugs is a logical extension of this hypothesis as, if the hypothesis is true, any drug which directly increases cyclic GMP levels in a smooth muscle should tend to lower intracellular ionised calcium levels and relax the muscle. However, in the experiments of Schultz *et al.*¹³ the effects of the relaxant drugs on cyclic GMP levels in the vas deferens were measured in the absence of extracellular calcium, and the effects of the drugs on muscle tension were not monitored. In our opinion, if direct correlations between cyclic nucleotide levels and drug-induced relaxation are to be made, it is essential that both parameters be measured. Therefore, the experiments described here were carried out in physiological salt solutions containing calcium, and the effects of relaxant drugs on both tension and cyclic nucleotide levels were monitored simultaneously in the same muscle segments.

In order to demonstrate the relaxant effects of drugs on the isolated vas deferens it was first necessary to contract the muscles. This was accomplished either by adding 30 μ M phenylephrine to the muscle baths or by exposing the muscles to 124 mM KCl¹⁰. The effects of these agents on tension developed by the muscles are shown in Fig. 1 together with the effects of verapamil and sodium nitroprusside on KCl- and phenylephrine-induced contractions. Verapamil at 20 μ M was capable of relaxing segments of rat vas deferens which had been previously contracted by KCl (Fig. 1 *a*) or by phenylephrine (data not shown). Pretreatment with 50 μ M verapamil for 30 s almost completely blocked the stimulatory effects of a subsequent dose of phenylephrine (Fig. 1 *b*). Similar results were obtained with hydralazine (not shown) although the onset of relaxation was slower than with verapamil and higher concentrations of hydralazine (1 mM) had to be used to obtain comparable degrees of relaxation. Sodium nitroprusside had no effect on contractions induced by phenylephrine (Fig. 1 *c*) or KCl (Table 1).

Cyclic nucleotide levels were determined in paired segments of rat vas deferens exposed to several combinations of stimulant and relaxant drugs. In agreement with previous reports⁸ cyclic GMP levels were markedly increased in segments of vas deferens exposed to KCl alone. For example, cyclic GMP levels were increased from 5.1 ± 1.6 pmol per g tissue in relaxed controls, to 31.9 ± 4.0 pmol per g tissue 5 min after KCl-induced depolarisation ($N=5$). The KCl-depolarised muscles could be relaxed by concentrations of hydralazine or verapamil which had no further effect on cyclic GMP levels (Table 1). These concentrations of hydralazine and verapamil were sufficient to completely relax the muscles within 6-10 min, although at 1 min after drug administration they were relaxed by only 16% and 38% respectively. Conversely, sodium nitroprusside failed to relax KCl-induced contractions of rat

Fig. 1 Representative tracings illustrating the effects of verapamil and sodium nitroprusside (SNP) on KCl- and phenylephrine-induced contractions of isolated rat vas deferens.

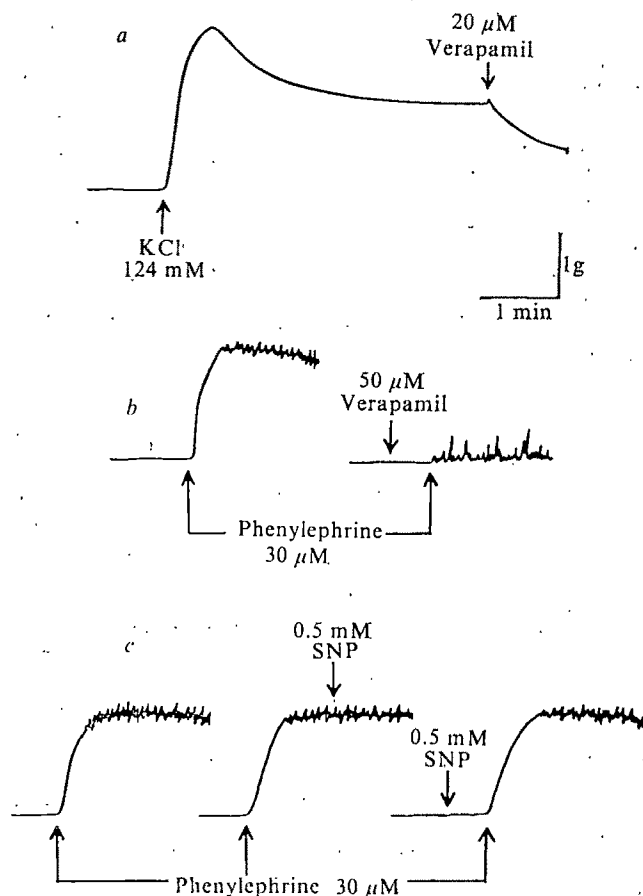


Table 1 Effects of hydralazine, verapamil and sodium nitroprusside on tension and cyclic nucleotide levels in rat vas deferens

Treatment	N	Cyclic GMP (pmol per g tissue)	Cyclic AMP (pmol per g tissue)	Relaxation (%)
KCl, 5 min (control)	5	33.0±1.5	683±74	—
KCl, 5 min+hydralazine last 1 min	5	33.6±2.6	658±31	16.4±1.5*
KCl, 5 min (control)	7	31.7±3.8	863±88	—
KCl, 5 min+verapamil, last 1 min	7	33.1±5.8	855±70	37.6±3.8*
KCl, 5 min (control)	6	30.2±6.5	621±138	—
KCl, 5 min+Na nitroprusside, last 1 min	6	67.8±13.8*	646±120	0.0
Phenylephrine, 2 min (control)	6	16.3±3.9	717±90	—
Phenylephrine, 2min+Na nitroprusside, last 1 min	6	120.5±19.6*	783±89	0.0

Paired segments of rat vas deferens were equilibrated in physiological salt solution as previously described¹¹. Both muscles of a given pair were then exposed to either 124 mM KCl or 30 µM phenylephrine for 5 or 2 min respectively. One muscle from each pair was also exposed to a relaxant drug for the last minute of the experiment. Concentrations of the relaxant drugs used were: verapamil, 20 µM; hydralazine, 1 mM; sodium nitroprusside, 0.5 mM in the phenylephrine experiments and 0.1 mM in the KCl experiments. Muscles were clamp-frozen at the appropriate times and cyclic nucleotides were extracted and assayed using standard techniques¹¹. Values represent means ± s.e.m. for the number of experiments indicated (N).

*Significantly different from corresponding controls ($P < 0.001$).

vas deferens, while significantly increasing cyclic GMP levels in these preparations (Table 1). Furthermore, 0.5 mM sodium nitroprusside, which had no effect on phenylephrine-induced contractions, increased cyclic GMP levels almost eightfold over the corresponding phenylephrine controls (Table 1). No significant changes in cyclic AMP levels were seen in any of the experiments. Thus, in the case of hydralazine and verapamil we were able to markedly relax the vas deferens without changing cyclic GMP levels, and in the case of sodium nitroprusside we were able to markedly increase cyclic GMP levels without relaxing the muscles. These results are not consistent with the suggestion that the relaxant effects of these drugs are mediated by increases in tissue levels of cyclic GMP.

It might be argued that the sodium nitroprusside-induced increases in cyclic GMP levels were activating mechanisms that tend to lower intracellular calcium, but that this effect was not sufficient to antagonise the contractile effects of phenylephrine or KCl. It should be noted, however, that both types of contractions could be relaxed by verapamil or hydralazine. The concentration of phenylephrine used in our experiments was submaximal, and phenylephrine-induced contractions could be almost completely blocked by pretreatment of the muscles for 30 s with relatively low concentrations of verapamil (Fig. 1). Cyclic GMP levels were not significantly altered by the verapamil pretreatment. Levels of the cyclic nucleotide were 15.8 ± 2.6 pmol per g tissue in the muscles exposed to phenylephrine alone and 11.3 ± 1.8 pmol per g tissue in muscles exposed to both verapamil and phenylephrine ($N=6$). Conversely, increases in cyclic GMP levels even larger than those shown in Table 1 were seen when the muscle segments were pretreated with sodium nitroprusside for 30 s before adding the phenylephrine. In these conditions cyclic GMP levels in the muscles exposed to both sodium nitroprusside and phenylephrine were increased more than 15-fold over the phenylephrine controls (from 15 ± 5 to 226 ± 27 pmol per g tissue, $N=3$). In spite of this large increase in cyclic GMP levels no detectable change in the phenylephrine-induced contractions was seen (Fig. 1). If cyclic GMP is in fact a mediator of relaxation, such a large increase in tissue levels of cyclic GMP should have at

least partially reduced the contractions caused by these submaximal concentrations of phenylephrine.

Our results indicate that increases in cyclic GMP levels are probably not responsible for the relaxant effects of sodium nitroprusside, hydralazine or verapamil in rat vas deferens. Further work is needed to clarify the role of cyclic GMP in smooth muscle relaxation.

This work was supported by USPHS grant HD-05827 from the National Institute of Child Health and Human Development. We thank Mrs Kathleen Anstiss for technical assistance.

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Arginine-specific reagents remove sodium channel inactivation

PROTEINS are often suggested to be the molecular components of excitable membranes which confer voltage-dependent permeability properties on nerve and muscle cells. Because of the relatively low concentration of the protein molecules directly responsible for ionic conductances compared with other membrane components, the isolation, characterisation, and reconstitution of these proteins is still at a rather early stage. Certain aspects of the molecular nature of the conductance mechanisms of excitable tissues, however, can be deduced using chemical reagents which specifically modify protein molecules. In particular, the sodium inactivation process can be eliminated by treatment with various proteolytic enzymes^{1–3,6}. Of these, trypsin is probably the most selective, cleaving mainly at arginyl or lysyl residues⁴. Several investigators have suggested that just such an exposed positively charged residue might be responsible for blocking the sodium conductance pathway to produce inactivation^{5,6}. Therefore, it should be possible to alter or remove inactivation by changing the configuration and/or charge of the blocking residue with a reagent specific for the residue involved. Glyoxal, phenylglyoxal and condensed 2,3-butanedione are three such reagents which are very reactive with the guanidino group of arginine^{7–9}. This paper describes the effect on sodium inactivation of these agents when they are internally perfused in the squid axon.

Two methods of internal perfusion were used: the 'roller' method¹⁰, and the 'cannula' method¹¹. In either case, axons were then mounted in a plexiglass chamber designed for voltage clamping using conventional techniques^{12,13}. Feedback compensation was used in all experiments to reduce errors due to membrane series resistance.

Glyoxal was allowed to react with the inner surface of the axonal membrane by perfusing the fibre for 10–15 min with a 5 mM solution of the trimeric dihydrate in an internal solution at pH 9.0. The pH 9.0 internal solution by

itself produced only a slight reduction in peak inward sodium current, with little (if any) effect on inactivation as estimated from the steady-state inward current at the end of a 10-ms pulse. At the end of the reaction period, the glyoxal had reduced the peak inward current to approximately 50% of its original control value, but the steady-state current at the end of the 10-ms pulse had increased by 200%. This result seems to reflect the complete removal of inactivation in affected sodium channels, as the time constant of decay in the component of current that still inactivated was unaffected by glyoxal treatment. Figure 1 shows the effect of glyoxal treatment.

To quantify this effect, a 25–50-ms conditioning prepulse was used to determine the relationship between inactivation and membrane potential before and after glyoxal treatment. In Fig. 2b, the normalised peak inward current is plotted against conditioning potential. In the control case, the peak inward current is rapidly reduced as the conditioning prepulse becomes more depolarised until, for large prepulses, more than 90% of the peak inward current is inactivated. After treatment with glyoxal, a portion of the peak sodium current was inactivated with a voltage dependence similar to the control, but a large fraction of the current is unaffected by the conditioning prepulse even at large depolarisations. We associate this fraction with the sodium inactivation molecules that have reacted with glyoxal.

Fig. 1 Current-time records before and after glyoxal. *a*, Family of current time records in the control solution: 500 mM CsF, 5 mM NaF and 20 mM CHES buffer adjusted to pH 9.0. The external solution was filtered seawater. The currents were produced by potential steps in increments of 10 mV from –30 mV to +90. *b*, Effect of 5 mM of the trimeric dihydrate of glyoxal (Sigma) applied in the control solution for 15 min. The number of voltage steps has been reduced for clarity. The currents shown are produced by potential steps in increments of 10 mV from –10 mV to +40. The experiment was carried out at the Marine Biological Laboratory, Woods Hole, Massachusetts, on axons of *Loligo pealei*.

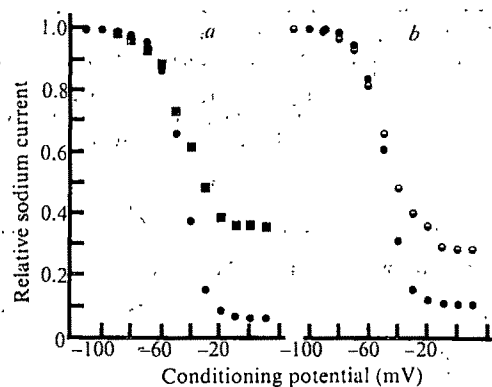
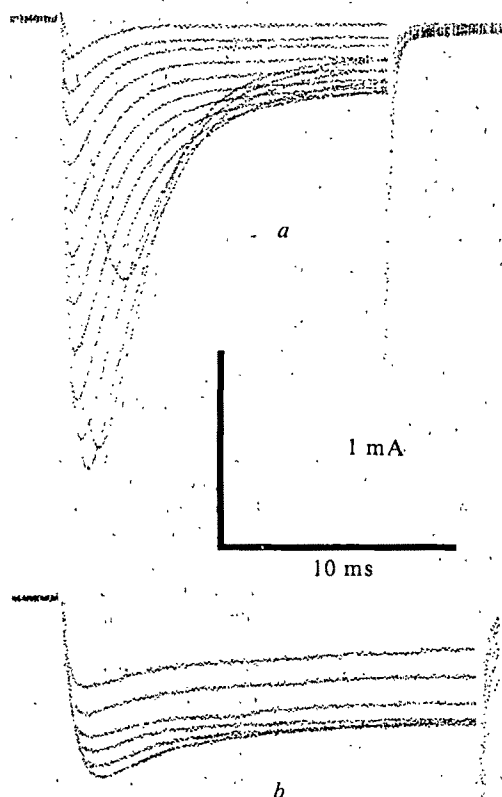


Fig. 2 Removal of inactivation by arginine-specific reagents. Thirty millisecond conditioning prepulses of various amplitudes were given before to a test pulse (+10 mV). The ratio of the test current for different conditioning potentials to the maximum test current at large negative conditioning potentials is plotted. *a*: ●, control values with an internal solution containing 400 mM KF, 100 mM TEABr, 5 mM NaF and 20 mM CHES buffer adjusted to pH 9.0; ■, same conditions after treatment with 15 mM diacetyl trimer for 45 min. *b*: ●, control internal solution was 500 mM CsF, 5 mM NaF and 20 mM CHES buffer to pH 9.0; ○, same conditions after treatment with 5 mM of the trimeric dihydrate of glyoxal. The trimer of 2, 3-butanedione was synthesised according to the method of Grossberg and Pressman¹⁴. A 15% aqueous solution of 2, 3-butanedione buffered to pH 8.0 with potassium phosphate was allowed to condense at constant pH for 48 h before use. This yields a solution of approximately 0.3 M trimer.

We also applied phenylglyoxal. A 1 mM solution of the reagent applied in a pH 7.3 internal saline produced a decrease in peak inward current without any removal of inactivation. Higher concentrations of phenylglyoxal led to a rapid and relatively complete removal of peak inward current, and a concomitant increase in leakage. Effects on inactivation in these conditions were difficult to determine.

Use of the third arginine reagent, 2,3-butanedione trimer, is complicated by the long reaction times necessary at pH 7.3. Fortunately, reaction at higher pH is more rapid^{9,14}. In squid, perfusing with a 15 mM solution of trimer in internal perfusate at pH 9.0 led to a 50% reduction in peak inward current and substantial removal of inactivation (Fig. 2a).

None of the effects of application of the reagents is reversible. The reduction of peak inward current for all the reagents and the removal of inactivation for glyoxal and the diketone trimer were not altered even after repeated changes of internal perfusion solution free of the reagents.

The previous results made arginine (or possibly lysine) a likely component of the Na⁺ inactivation mechanism. To test the possibility that arginine might actually be the blocking residue of the inactivation molecule, we completely removed inactivation by treatment with Pronase², and then

Table 1

Amino acid	Effect	Fraction of sodium* current after treatment
Arginine	+	0.76 ± 0.07 (n = 6)
Lysine	0	0.96 ± 0.06 (n = 6)
Aspartic acid	0	1.00 (n = 1)
Glutamic acid	0	1.00 (n = 1)
Glycine	0	1.03 ± 0.06 (n = 3)
Histidine	0	1.08 ± 0.07 (n = 4)
Tyrosine†	0	1.01 (n = 2)
Asparagine	0	0.99 (n = 2)
Glutamine	0	0.99 ± 0.07 (n = 4)

Internal solution contained (mM): CsF 500, HEPES 20, Na 5 + amino acid 50. External solution was filtered seawater.

*Ratio of inward current in the presence of the amino acid to the inward current in the absence of the amino acid (for a voltage step to 0 mV).

†Tyrosine was applied as a 5 mM solution.

internally applied various amino acids. Table 1 shows that of the amino acids tested only arginine shows a significant blocking ability. Notably, lysine produces no significant blocking effect. We feel that the reason arginine produces a simple blockage of current rather than a time-dependent reduction of current similar to normal inactivation is due to the relatively mobile character of the free amino acid. This blockage is analogous to the effect of tetraethylammonium (TEA) and its higher analogues on the sodium channel⁶. TEA equilibrates with the sodium conductance mechanism so rapidly that no time dependence is observed. TEA analogues with a hydrophobic side chain equilibrate at a much slower rate, producing a time dependent reduction in current.

With this analogy in mind, the possibility that arginine could be the blocking residue was tested further by internally applying the polypeptide, polyglycyl arginine amide,

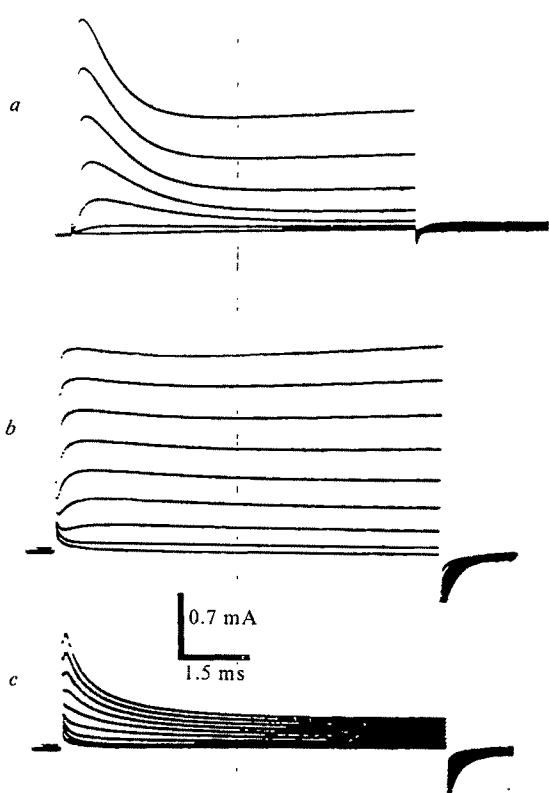


Fig. 3 Effect of polyglycyl-*N*-acetyl arginine amide on outward sodium currents in a fibre, without inactivation. Axon bathed in Tris-HCl 450 mM, MgCl₂ 50 mM, CaCl₂ 10 mM, pH 7.9; internally perfused with 250 mM NaF, 500 mM sucrose, 5 mM Tris-HCl, pH 7.3; temperature 5 °C. The figure shows currents in response to voltage clamp pulses that took the membrane potential to the values indicated below from a holding potential of -60 mV. *a*, Current records for pulses to -30, -10, 10, 30, 50, 70 and 90 mV. *b*, Current records after perfusing the axon for 12 min with Pronase dialysed overnight against EGTA (final concentrations: Pronase, 1 mg ml⁻¹, EGTA 1 mM). Membrane potential during the pulses: -30, -10, 10, 30, 50, 70, 90, 110 and 130 mV. *c*, Seven minutes after the records in *b* were taken, the axon was internally perfused with polyglycyl-*N*-acetyl arginine amide (approximately 0.1 mM molecular weight 10,000) and the records shown taken 5 min later. Membrane potential during the pulses: -30, -10, 30, 50, 70, 90, 110 and 130 mV. The polyglycyl-*N*-acetyl arginine amide was synthesised by coupling polyglycine of molecular weight 5,000 to 10,000 (Sigma) with *n*-acetyl-arginine (Sigma) using a water-soluble carbodiimide as a coupling agent¹⁸. Separation of the desired peptide from unreacted components was accomplished by chromatography in Sephadex G-10, followed by ion exchange in CM-Sephadex. The experiment was carried out using axons of *Loligo forbesi* at the Marine Biological Association, Plymouth, UK. Similar results were obtained with a longer polyglycyl arginine derivative (molecular weight 20,000).

in an axon which had previously been treated with Pronase to remove inactivation. The results suggest that this peptide can mimic sodium inactivation (Fig. 3).

In axons treated with Pronase, perfused internally with sodium fluoride containing the peptide and bathed in sodium-containing artificial seawater, time dependent block of both the inward as well as the outward sodium currents was observed (E. Rojas and B.R., unpublished).

When considering these results, special attention must be given to the specificity of the reagents used to react with the presumptive arginyl residue. For each reagent used, the only significant reaction besides that with guanidino moieties is with amino groups. The reaction of all the reagents with amino groups is much slower and less complete than with arginine⁷⁻⁹.

Thus, the reaction kinetics of the reagents make reaction with lysine seem less likely than arginine. If the reagents are actually attacking a blocking residue acting as either a physical or electrostatic 'plug' during inactivation, the results of the amino acid and polypeptide application make the reaction with arginine even more attractive since lysine seems to be unable to block the sodium channel.

The reduction in the peak inward current after application of the reagents may be due to several things. Of course, the reagents may be directly reacting with the sodium conductance mechanism in some unspecified way. Alternatively, each of the reagents may be capable of cross-linking reactions¹⁸. This might lead to an occasional reaction where the blocking residue of the inactivation molecules is cross-linked while in a blocking position, with a consequent reduction in sodium conductance.

We can consider these results in the context of the models already suggested^{5,6}. Both models require a charged group that is 'tethered' to the membrane. The charged group is capable of blocking open sodium channels and consequently producing inactivation. In the model of Rojas and Rudy, it is strongly suggested that the charged moiety is an arginyl residue in a peptide chain. Our data with the amino acids and polypeptide suggest that an arginyl residue could actually be the blocking component, but that lysine could not.

But, whatever the actual mechanism of inactivation, our results with glyoxal and diacetyl trimer strongly suggest that an arginine residue must be an important functional part of the inactivation process.

This work was supported by a grant from the NIH (NS-11963).

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Received 28 November; accepted 19 December 1977.

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Photoreceptor electric potential in the phototaxis of the alga *Haematococcus pluvialis*

THE nature of the primary processes in the photoreceptor and the mechanism of their coupling with the mechanical response of the flagella are the most important and, at the same time, the least investigated problems of the phototaxis in flagellates¹⁻⁵. It has been suggested that the transduction of the light stimulus in phototaxis might involve bioelectric processes^{4, 6-9}, but no direct experimental evidence supports this hypothesis. The bioelectric control of the locomotor responses to light^{10,11}, mechanical¹² or electrical¹³ stimulation has been found in ciliates, but they do not possess the orientated phototactic response mechanism¹⁴ of flagellates. Now we demonstrate the photoinduction of electric potentials related to phototaxis in the unicellular flagellated alga *Haematococcus pluvialis*. Photo-stimulation evokes a graded receptor potential which gives rise to a regenerative potential dependent on the presence of extracellular calcium ions.

Insertion of a microelectrode rapidly damages the cell, hence electrical potentials were registered extracellularly. In the developed technique the cell was sucked (under microscopic control) into a tip of a glass micropipette (Fig. 1), causing two parts of the cell surface, the inside and outside, to become electrically isolated by the glass of the pipette (shunt resistance, up to 100 MΩ) thus enabling measurement of the electrical potential difference (PD) between them. The micropipette and the chamber in which its tip was located were filled with the same solutions (usually growth medium) and connected by KCl bridges and calomel electrodes to the amplifying and registering equipment similar to that described earlier^{15,16}. A signal was considered positive if the inner part of the pipette had a positive potential in reference to the potential of the chamber. The time resolution of the system was about 1 ms.

For actinic illumination, a flash xenon lamp (flash duration, less than 1 ms) or a halogen incandescent lamp provided with an electrical shutter (opening time, about 1 ms) were used. The intensity of a light stimulus was measured by a thermopile or a calibrated photodiode and was changed from 10² to 10⁶ erg cm⁻² s⁻¹ by neutral light filters. The measurements were carried out at room temperature using a water infrared filter.

The cells of *H. pluvialis* were grown at 20° C under constant illumination with fluorescent lamps (500 lx) in a medium containing 1 mM KNO₃, 0.4 mM K₂HPO₄, 0.3 mM MgSO₄, 0.3

mM Ca(NO₃)₂, and a standard solution of trace elements, and 3-7-d-old cultures were used.

The sucking of a cell into a micropipette leads to its slight deformation, but even after a 2-3-h experiment the cell regained its normal motility and a phototactic ('stop') reaction after being released from the pipette. In the dark or under red light illumination, there is no PD between the chamber and the inside part of the micropipette separated by the cell. On 1 ms flash illumination with white or blue-green light, a fast transient PD between the two parts of the cell surface (inside and outside the pipette) is generated (Fig. 2a). This primary photoinduced potential difference, PPD, comprises a fast rise of PD and its slower drop to zero. The response is graded, both the amplitude and the rate of the potential changes rising with the increase of light intensity.

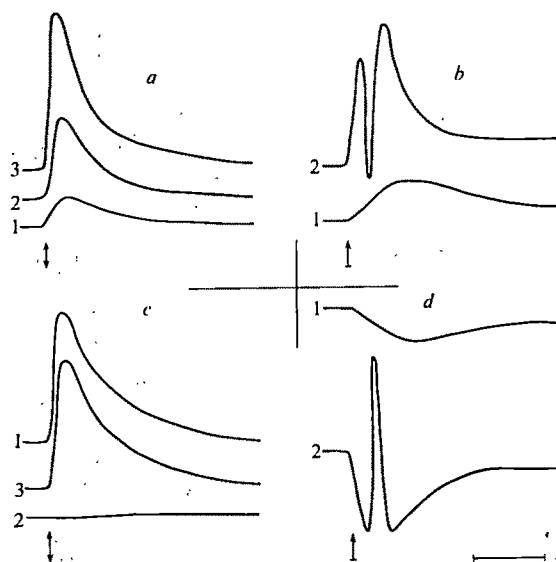
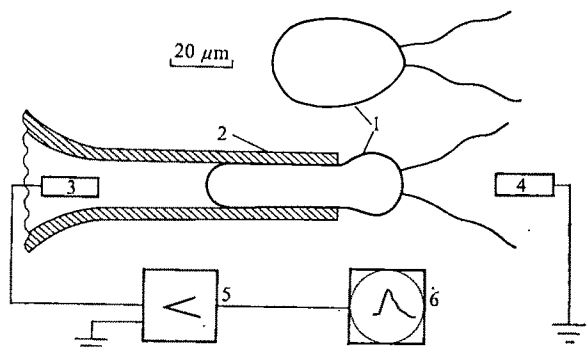


Fig. 2 Kinetics of photoinduced electric potential difference (PD) between the two parts of the cell surface, the inside and outside of the pipette. The arrows mark the moment of a flash (for *a* and *c*) or of a switching on of continuous light (for *b* and *d*). An upward movement of the traces means an increase in potential inside the micropipette. Calibration: 2 mV and 100 ms. *a*, The posterior part of the cell is inside the pipette for *a*, *b*, *c*. Primary PD induced by 1 ms flash of three different intensities (1, 5 and 12 relative units for curves 1, 2 and 3, respectively). *b*, PD induced by continuous light of different intensity (1 and 8 relative units for curves 1 and 2, respectively). At the intensity exceeding a threshold level, primary PD becomes superimposed by a secondary regenerative response (curve 2). *c*, Effect of 3 mM azide on photoinduced PD. 1, Before the addition of azide; 2, 30 s after azide addition; 3, after the removal of azide from the chamber by a fresh solution. *d*, The same as *b*, but the anterior part of the cell is inside the pipette.

Fig. 1 Scheme of a cell fixation in a suction micropipette and extracellular potential difference registration. 1, Cell; 2, tip of the micropipette; 3, calomel electrode connected to the interior of the micropipette; 4, calomel electrode connected to the chamber; 5, amplifier; 6, oscilloscope.



When the intensity and/or the duration of a light stimulus exceed some threshold, the PPD becomes superimposed by a regenerative electric response (RR) (Fig. 2b). This spike-like signal has a two-wave form and usually one of the waves (positive or negative) dominates. Its amplitude reaches a constant level within a narrow range of rising stimulus intensities. Further light intensity increase leads to the shortening of the lag-period of RR from 60 ms to 5 ms. The blue-light induced RR has a refractory period of about 0.5-1.0 s. The appearance of the blue-light induced spike can be stimulated by the red background illumination or by the increase of the frequency of blue flashes (from 1 to 20 per min), while the prolongation of a dark period leads to the disappearance of RR. These features of the spike-like electrical signal allow its separation from PPD.

RR is sensitive to poisoning by heavy metals or to the removal of Ca²⁺ ions by EDTA. Both factors also affect PPD, but to a much lesser degree. Spontaneous spikes can be sometimes

observed in the dark or under constant illumination. Their complex kinetics is similar to that of the photoinduced signals.

We believe that RR reflects the excitation of the whole cell membrane in a similar way, perhaps, to that found in ciliates^{11,13}. It is noteworthy that removal of Ca^{2+} also blocks the phototactic response (stopping, reversed locomotion) of *Chlamydomonas*⁹. Thus the RR is likely to play a significant part in phototaxis.

PPD has no (or less than 1 ms) lag-period. Its initial rise rate depends linearly on the light intensity up to $10^8 \text{ erg cm}^{-2} \text{ s}^{-1}$, the rise time being not more than 5 ms at this intensity. In contrast to this, the amplitude of the signal has a nonlinear light dependence and a tendency to saturate at high intensities. The PPD signals can reach 5–7 mV and can be reversibly inhibited by azide (Fig. 2c).

It was found that the sign of PPD is determined by the orientation of the cell in the pipette. The positive signals were registered in all cases when the posterior or lateral part of the cell was sucked into the pipette (Fig. 2a, b, c). If the same cell was fixed in the pipette so that the anterior, flagella-bearing part was in the pipette, signals of negative polarity but similar form were observed (Fig. 2d). This observation suggests that the anterior zone of the cell becomes negatively charged with respect to the other part of the cell surface under the action of light.

It is natural to suggest that the photoinduced polarisation of the cell is due to the asymmetrical location of the photoreceptor (an electric potential generator) in the cell membrane. Taking into account that the negative potential can be registered only from a small part of the anterior cell surface, one may conclude that the photoreceptor is located in this part of the cell, has a small size and generates the positive potential inside the cell with respect to the outer space under the influence of light. A simple equivalent scheme of the extracellular measurements of the potential can be proposed (Fig. 3). The scheme does not have capacity elements and cannot be used to describe the kinetics of the signals.

The anterior (a) and posterior (p) parts of the cell with transmembrane resistances R_a and R_p , respectively, are externally separated by the shunt resistance R_{sh} . The resting potentials (E_m) of the anterior and posterior membranes compensate one another in the dark. On illumination, the photoreceptor in the anterior part of the cell membrane generates an electrical potential E_r which is measured across R_{sh} as the PD. E_r can be determined if R_{sh} , PD and R_p are known. It was found that the light-induced voltage rises linearly with increase in shunt

Fig. 3 Simple equivalent scheme of the extracellular registration of the transmembrane receptor potential (see text). p and a, Posterior and anterior parts of the cell surface (inside and outside the pipette); R_p and R_a , the resistances of the posterior and anterior parts of the cell membrane; E_m , transmembrane potential in the dark; E_r , photoinduced receptor potential; R_{sh} , shunt resistance between p and a; G, voltmeter.

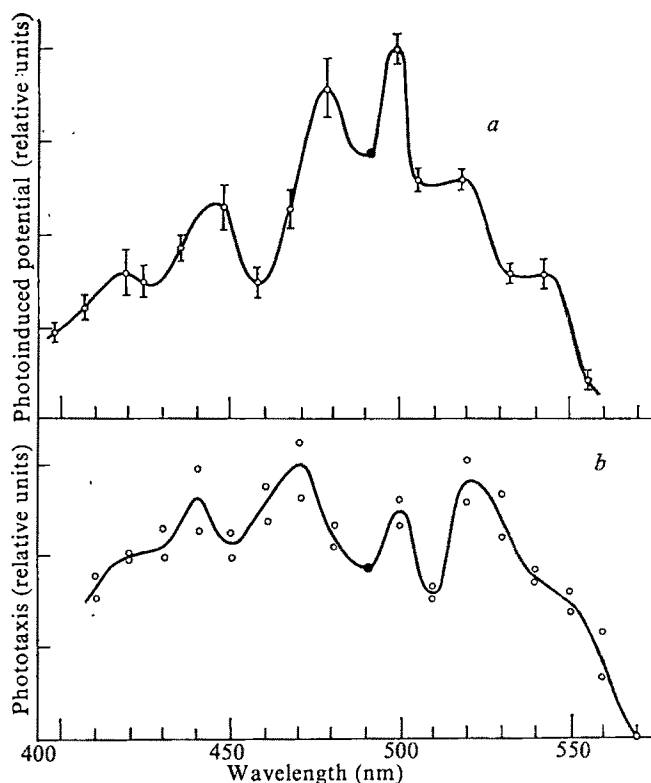
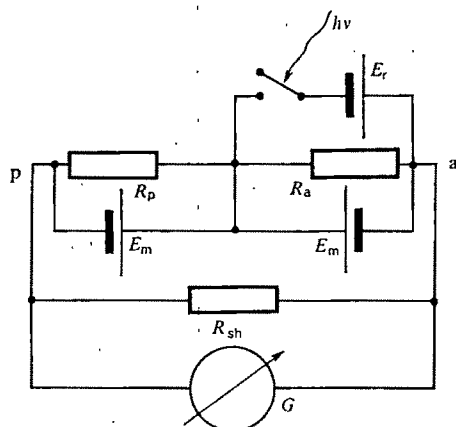


Fig. 4 Action spectra for the initial rate of PD rise (a) and the rate of phototactic cell accumulation in the lighted region (b). Spectral sensitivity at each wavelength was compared to that at 490 nm. Each point on curve a represents a mean value of 6–12 measurements $\pm \sigma$ corrected for the number of incident quanta. Spectrum b was measured under the conditions of equal no. of incident quanta at each wavelength.

resistance (measured as a difference between the resistance of the pipette with the cell and that without the cell) as the cell is sucked into the pipette. With R_{sh} of $10^8 \Omega$, PD is 3.5 mV. Assuming that the specific membrane resistance of the cell membrane has an average value of about $2 \times 10^4 \Omega \text{ cm}^2$ (refs 17, 18), R_p is $1.4 \times 10^9 \Omega$ for a spherical cell 30 μm in diameter. For these values, the light-induced transmembrane potential E_r is about 50 mV. Because of the changes in the geometry of the cell in the pipette and of the possible deviations of the value of the specific membrane resistance from the accepted average, this calculation of E_r is a rough estimate.

It was found that only the blue-green region of the spectrum is efficient in the generation of the described potentials. The red and infrared light ($\lambda > 630 \text{ nm}$) have no effect, even at the intensities 3–4 orders of magnitude higher than the usually effective intensities of the blue light. The action spectrum for the rate of the PPD rise was measured with the use of interference filters (band pass, 10 nm) and was compared to the action spectrum of phototaxis of this organism (Fig. 4). For the net phototaxis action spectrum measurements, the equipment similar to that described earlier by Lindes *et al.*¹⁹ was used. The kinetics of the cell accumulation in the actinic zone was monitored by a testing (measuring) beam ($\lambda = 750 \text{ nm}$). The intensity of the monochromatic actinic light at each wavelength (band pass, 5 nm) was measured and equalised in a number of incident quanta before each kinetic curve was recorded. The initial rate of the cell accumulation in the illuminated zone was taken as a criterion of phototaxis (phototactic index)²⁰. As seen from Fig 4, the action spectra of the PPD generation and the net phototaxis are similar. This points to the generation of the described potential by the receptor pigment(s) of phototaxis.

Rapid (less than in 30 s) and reversible inhibition of the electrical signal by azide (3 mM), known as an efficient and reversible inhibitor of phototaxis²¹, also indicates that there is

close connection between the potential generation and phototaxis. PPD, however, is not sensitive to 3-(3,4-dichlorophenyl)-1,1-dimethylurea, the inhibitor of photosynthesis, in usually efficient concentrations (10^{-5} M).

The time characteristics of the electrical signal, its dependence on the intensity and spectral composition of the actinic light, the localisation of the photoreceptor, the character of the inhibitors' action, and others suggest that the photoinduced potential is a photoreceptor potential of phototaxis. The mechanism of the potential generation is not yet clear, and may be based on changes in permeability for ions, as well as active electrogenic processes. Also, the receptor potential may affect the action of flagella directly or through the secondary regenerative response. To solve these problems further experiments are necessary.

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Received 22 August; accepted 16 November 1977.

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Stimulation of brain membrane protein phosphorylation by calcium and an endogenous heat-stable protein

THE important role of Ca^{2+} in the physiology of the nervous system is well documented^{1,2}. However, the biochemical mechanisms underlying certain of its physiological effects, such as stimulus-secretion coupling^{3,4} and synthesis of catecholamines^{5,6}, have not yet been elucidated. Calcium has been implicated in several biochemical reactions of potential importance to synaptic function. Thus, calcium and a heat-stable calcium-binding protein activate the cyclic nucleotide phosphodiesterase from mammalian brain^{7,8}. The calcium-dependent regulator (CDR) from porcine brain⁹, bovine heart¹⁰ and bovine brain¹¹ has been purified and characterised. CDR seems to be a calcium receptor as it binds calcium strongly and specifically. There is evidence that a CDR· Ca^{2+} complex is the true activator of cyclic nucleotide phosphodiesterase¹²⁻¹⁴. A detergent-solubilised preparation of brain adenylate cyclase can also be activated by this calcium-binding protein¹⁵. Calcium stimulates protein phosphorylation in both intact^{16,17} and lysed^{18,19} synaptosomes and this cyclic nucleotide-independent mechanism may mediate or modulate some of the intracellular effects of Ca^{2+} on the function of presynaptic nerve terminals. We report here that calcium-dependent phosphorylation of synaptosomal membrane fractions from rat cerebral cortex requires an endogenous protein factor present in the synaptosomal cytoplasm. Calcium stimulated phosphorylation is lost on purification of synaptic membranes and can be effectively recovered by reconstitution with either the synaptosomal cytoplasm or with a purified preparation of CDR. Thus, regulation by calcium of calcium-dependent protein kinase activity

may be mediated physiologically by the calcium-binding protein postulated to regulate cyclic nucleotide phosphodiesterase and adenylate cyclase.

The effect of calcium on endogenous protein phosphorylation in lysed synaptosomes and in synaptic membranes is shown in Fig. 1. Synaptosomes obtained from rat cerebral cortex were lysed by hypo-osmotic shock and assayed for incorporation of ^{32}P -phosphate from γ - ^{32}P -ATP into protein (Fig. 1, lanes 1 and 2). Calcium caused a marked stimulation of endogenous protein phosphorylation. A net increase in ^{32}P -phosphate incorporation into a number of protein bands was observed, with those having molecular weights of about 51,000 and 62,000 showing highest levels of incorporation. Similar patterns of stimulation by calcium have been observed by DeLorenzo and Freedman^{18,20}, though much higher calcium ion concentrations seem to be required in their system.

The calcium-dependent phosphorylation observed in assays containing both synaptic membrane fractions and synaptosomal cytoplasm (that is, lysed synaptosomes) is lost on

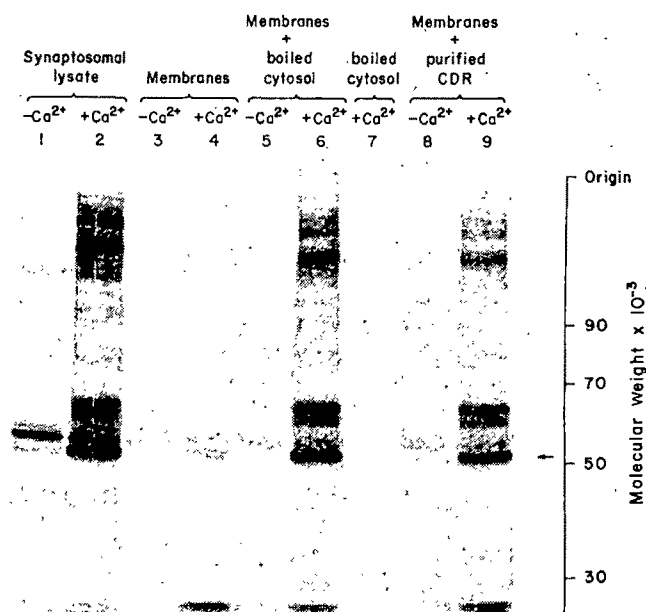


Fig. 1 Effect of Ca^{2+} , synaptosomal cytoplasm, and purified CDR on endogenous protein phosphorylation of brain membranes. Male Sprague-Dawley rats (150-200 g) were killed by decapitation and the cerebral cortex used for preparation of synaptosomes (P2) by minor modification of the method of Gray and Whittaker²⁸. The suspension of synaptosomes obtained from 2 g of cerebral cortex was subjected to osmotic shock by homogenisation in 10 volumes of ice-cold water (15 ml) and kept on ice for 30 min to optimise lysis. The synaptosomal lysate contained 2.5 mg protein ml^{-1} . Total membranes were obtained from lysed synaptosomes by centrifugation at 150,000g for 30 min. The released synaptosomal cytosol (0.7 mg ml^{-1}) was removed, heated at 95-100 °C for 5 min, and used as the source of activator. The pellet was resuspended in 15 ml of 5 mM Tris-HCl (pH 7.0), centrifuged as above, and again resuspended in 15 ml of 5 mM Tris-HCl (pH 7.0). This resuspended pellet (1.7 mg ml^{-1}) served as the source of activator-deficient membranes. Membranes stored at -20 °C were stable for about 1 month. CDR was purified to homogeneity by the method of Teo *et al.*¹⁰. The reaction mixture for assay of endogenous protein phosphorylation (final volume, 100 μl) contained: 50 mM PIPES buffer (pH 7.0), 10 mM MgCl_2 , 1 mM dithioerythritol, 0.2 mM EGTA (minus calcium) or 0.2 mM EGTA+0.5 mM CaCl_2 (plus calcium), 5 μM γ - ^{32}P -ATP (3.0 - 7.0×10^4 c.p.m. pmol^{-1}), and, where indicated, 50 μg lysed synaptosomes, 34 μg membranes, 14 μg boiled cytosol and 0.25 μg CDR. The reaction was initiated by addition of the γ - ^{32}P -ATP after preincubation for 30 s at 30 °C. Incubation was carried out for 10 s, the reaction terminated by addition of 50 μl of an 'SDS-stop solution', and a 75 μl aliquot of the sample analysed by SDS-polyacrylamide gel electrophoresis and autoradiography as described previously²⁹. Using procedures described elsewhere¹⁷, radioactive bands were found to be due to incorporation of ^{32}P -phosphate into protein (by phosphomonoester linkage) rather than into lipid or nucleic acid. Results similar to those shown here were obtained when more purified preparations of synaptosomes were used.

preparation of cytoplasm-free membranes (Fig. 1, lanes 3 and 4). This phenomenon was not due to inactivation of the calcium-dependent kinase activity during experimental manipulations as it could be regained by addition of an amount of synaptosomal cytoplasm (Table 1) or boiled synaptosomal cytoplasm (Fig. 1, lanes 5 and 6; Table 1) commensurate with the amount of membrane protein present. The heat-stable factor in the cytosol which conferred calcium-dependent phosphorylation on washed membranes did not exhibit any endogenous phosphorylation (Fig. 1, lane 7), nor did it show any histone, protamine or casein kinase activity (data not shown).

The heat stability of the stimulating factor in the cytosol suggested a possible relationship to the calcium-binding protein that modulates activities of cyclic nucleotide phosphodiesterase and adenylate cyclase. Therefore, this calcium-dependent regulator was purified to homogeneity from bovine cerebral cortex by the method of Teo *et al.*¹⁰, in order to test its effect in the calcium-dependent protein phosphorylation system. Addition of this protein to washed synaptosomal membrane fractions restored calcium-dependent protein phosphorylation (Fig. 1, lanes 8 and 9). The pattern of phosphorylation obtained on addition of heat-treated synaptosomal cytoplasm was indistinguishable from that obtained on addition of CDR, suggesting that the two factors are functionally equivalent.

Stimulation of protein phosphorylation was dependent on the presence of both calcium and either the cytosol factor or CDR. Thus, calcium in the absence of boiled cytosol and boiled cytosol in the absence of calcium were ineffective, whereas addition of both calcium and boiled cytosol restored phosphorylation (compare lanes 3, 4, 5 and 6). Analogous results were obtained with CDR (compare lanes 3, 4, 8 and 9). The endogenous activator, like CDR, is extremely heat stable, non-dialysable, resistant to DNase and RNase, and sensitive to trypsin (Table 1).

CDR is present in synaptosomal cytosol of bovine and rat cerebral cortex and should, therefore, account for some of the activation seen by reconstituting membranes with synaptosomal cytosol. However, this does not exclude the possibility that other factors also present in the cytosol may be physiologically important regulators of the calcium-dependent phosphorylation reaction. Therefore, we have recently purified a protein from bovine cerebral cortex based on its ability to stimulate protein phosphorylation in activator-depleted membranes (in preparation). Only a single peak of activity was present throughout the purification procedure. This activity co-purified with the phosphodiesterase activator, suggesting that this endogenous

activator of the calcium-dependent phosphorylation system is CDR.

The calcium-dependent regulator seems to have a rather broad distribution in the animal kingdom²¹. Various mammalian and invertebrate tissues have been found to contain low levels of calcium-activatable phosphodiesterase and very high levels of CDR²¹⁻²⁶, leading many investigators to speculate that there must be other functions for this protein. Indeed, Kretsinger has postulated that all of the processes in which calcium functions as a second messenger are mediated by calcium-binding proteins with binding properties similar to those of CDR²⁷. The findings reported here demonstrate that calcium and CDR can affect protein phosphorylation directly, by stimulation of a calcium- and CDR-dependent protein kinase system, and suggest that this system may mediate some of the presynaptic actions of calcium. Calcium and CDR can presumably also regulate protein phosphorylation indirectly, by changing cyclic nucleotide levels by means of effects on cyclic nucleotide phosphodiesterase and adenylate cyclase activities.

This work was supported by the USPHS and Jane Coffin Childs Memorial Fund for Medical Research. H.S. is a fellow of the Jane Coffin Childs Memorial Fund for Medical Research.

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Received 20 October; accepted 2 December 1977.

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Table 1 Characterisation of the cytosol factor

Addition to activator-depleted membranes	³² P-phosphate incorporated % of control
None	27
Untreated cytosol	100
Heat-treated cytosol	95
Dialysed cytosol	99
DNase-treated cytosol	99
RNase-treated cytosol	97
Trypsin-treated cytosol	28

Cytosol (14 µg) was preincubated in the presence of 50 mM Tris-HCl (pH 7.2), 1 mM MgCl₂ and, where indicated, DNase (50 µg ml⁻¹), RNase (50 µg ml⁻¹) or trypsin (10 µg ml⁻¹), in a total volume of 30 µl, for 60 min at 30 °C. Trypsin inhibitor (egg white) was then added at a final concentration of 25 µg ml⁻¹ to the trypsin treated sample. The cytosol factor remaining after these treatments was assayed for its ability to support endogenous protein phosphorylation of synaptic membranes (34 µg) using the reaction mixture described in the legend to Fig. 1. For quantitative determinations, the radioactive band at a molecular weight of 51,000 (see arrow, Fig. 1) was localised by autoradiography, cut out of the dried gel and counted by liquid scintillation spectrometry. Incorporation in the presence of untreated cytosol is taken as 100% and represents 17.3 pmol phosphate per min per mg membrane protein. DNase, RNase and trypsin + inhibitor (egg white) added to synaptic membranes without cytosol had no effect. Results qualitatively similar to those shown here were obtained with several other proteins bands.

The use of *Xenopus* egg cells to assay the mRNA of single cells

TRANSLATION of mRNA in egg cells and oocytes of *Xenopus laevis* Daudin has been shown to be very efficient for mRNAs from a variety of cells¹⁻⁷ and has uses in developmental biology, molecular biology and immunology⁸. We have also reported its use in cancer research⁹. One of the main disadvantages of this tool, however, is the fact that fairly extensive RNA extraction and purification procedures are required. In particular, in cases when different cell types are mixed, as in the case with immunologically active cells, it is almost impossible to obtain information about the activity of the separate cell types. Studies in this field are always dealing with, at best, highly enriched cell populations. Therefore we have looked for a method which avoids RNA extraction and purification, with the aim of being able to work with separate cells. We have tried to inject cell homogenates

directly into egg cells of *Xenopus*, and in the experiments reported here, we chose myeloma cells (LOU/M/WS1 rats with myeloma IR2 synthesising immunoglobulin E), because we could analyse the translation products easily and we could start with a relatively homogeneous cell population. Our results suggest that it is possible to study the translation products of single myeloma cells using this technique.

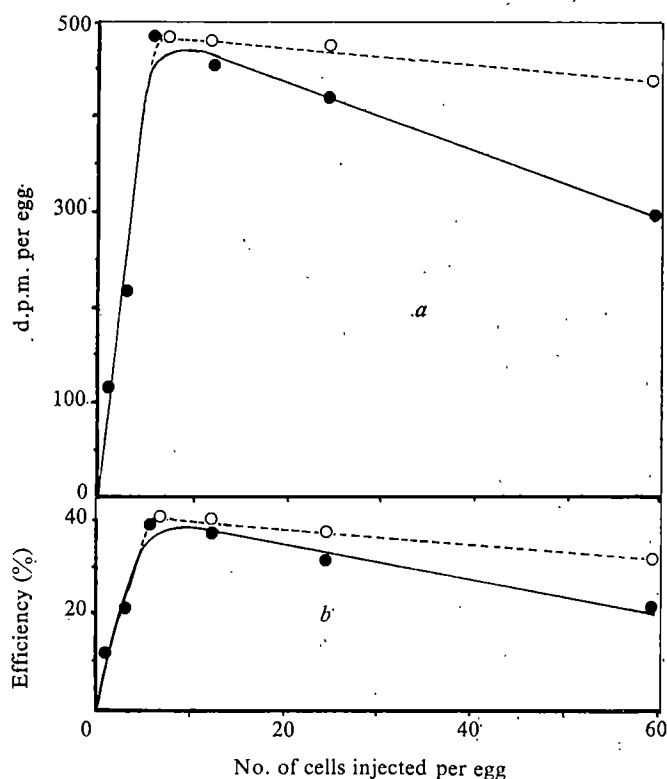


Fig. 1 *In ovo* synthesis of rat IgE. Female LOU/M/WS1 rats were infected with 0.5 ml myeloma IR2 cells (IgE-secreting myeloma¹⁰). After 7–9 d ascites was tapped and on average it contained 12.6×10^6 cells per animal. These were diluted 1:1 with Earle's balanced salt solution and layered on top of 5 ml Ficoll–Isopaque ($d=1.078$). After centrifugation for 30 min at 1,500g at room temperature¹¹, lymphocytes were taken from the top of the Ficoll–Isopaque cushion and washed twice with Earle's BSS. After the second washing, the cell suspension was divided into two equal parts, one for direct injection and one for RNA extraction. Both parts were frozen in solid CO₂ and used for injection and extraction the next day. Obtaining and handling of the egg cells of *Xenopus laevis* was as described earlier⁷, the only difference being the absence of actinomycin D in the incubation medium. Myeloma cells were suspended in double-concentrated injection medium, counted and homogenised. The final cell concentration corresponded to 5×10^6 cells ml⁻¹. One aliquot was diluted 1:1 with ³⁵S-methionine (Radiochemical Centre, Amersham, UK; specific activity 25 mCi mmol⁻¹) and 50 nl injected, containing 3×10^5 d.p.m. = 5 pmol methionine. A number of dilutions was then made of the cell homogenate (1:2, 1:4, 1:10, 1:20, 1:40, 1:125). Of each sample, 50 nl was injected per egg cell after 1:1 dilution with radioactive precursor. As a control, 50 nl of 1:1 diluted precursor was injected alone. RNA was extracted as described previously¹², yielding 0.16 ng total RNA per myeloma cell. Eggs were incubated for 16 h at 17 °C and homogenised in 400 µl PBS. The homogenate was taken up in a total of 800 µl PBS and centrifuged 30 min at maximum speed in a Spinco 50Ti rotor at 4 °C. To 100 µl of the clear supernatant 5 µl absorbed goat anti-rat IgE was added. After incubation at 37 °C for 15 min, 100 µl of rabbit anti-goat IgG was added and the mixture was incubated at 37 °C for a further 15 min. After overnight precipitation at 4 °C the pellets were spun down in an Eppendorf centrifuge 3200 and washed thrice with cold PBS. The final pellet was suspended in 100 µl of a buffer containing 0.1 M Tris-glycine pH 8.3, 2% SDS, 5 mM 2-2-mercaptoethanol, and counted on Whatman 3 MM filter paper disks after drying in a Nuclear Chicago Liquid Scintillation Counter (efficiency ~ 70%). *a*, Radioactivity precipitable with anti-IgE after subtraction of the control value (300 d.p.m.). ●, Disrupted cells injected; ○, Crude RNA injected. In addition to the specific precipitation, a total, 10% trichloroacetic acid (TCA) precipitate of every sample was counted. *b*, Efficiency of translation expressed as the ratio of d.p.m. precipitable with anti-IgE over d.p.m. precipitable with 10% TCA. All experiments were done in triplicate.

The result of injecting several dilutions of cell homogenate are given in Fig. 1. Comparison of these results with those obtained after injection of a cold phenol RNA preparation shows no difference in response at greater dilutions. We conclude, therefore, that *in ovo* synthesis of IgE continues at the same rate following injection of RNA or cell homogenate of equal cell numbers, up to a maximum at about ten cells. At higher concentrations, however, a large decrease in specifically precipitable radioactivity is found after injection of cell homogenate, whereas this amount is almost constant after injection of RNA. The decrease is not caused by a general inhibition of translation by substances in the cell homogenate—as confirmed by plotting the efficiency, expressed as the ratio of radioactivity precipitable with anti-rat IgE to TCA-precipitable radioactivity (Fig. 1*b*). Clearly, only the specific translation is inhibited at increasing cell number. Purified, deproteinised RNA does not show inhibition to this extent. Therefore, this phenomenon may depend on protein(s). The inhibitors do not interfere with the translation machinery itself, since the total TCA-precipitable radioactivity is unaffected. The results show the presence in the myeloma cell homogenate of a substance(s) that specifically inhibits the translation of injected RNA. The source of this inhibitory material is uncertain, but the fact that greater concentrations of cell homogenate are required to effect inhibition could indicate that it is derived from other than myeloma cells. Since our cell homogenate was prepared from cells in the ascites, it is also very likely to contain material from other cells. We did not determine which cell type could be responsible for the observed inhibition.

Maximum efficiency of translation is found after injection of the content of about ten cells. In another study with myeloma cells, saturation of the *Xenopus* translation machinery was found after injection of 0.9 ng A-rich-containing RNA⁸. If we assume that there was the same percentage of A-rich-containing RNA in the myeloma cells that we used, saturation in our experiments was reached after injection of comparable amounts of RNA.



Fig. 2 Electrophoretic analysis of the translation products of *a*, endogenous *Xenopus* mRNA and *b*, mRNA from IR2 myeloma cells. The position of unlabelled marker IgE (ref. 13), run in the same gel, is also indicated (*c*). Before electrophoresis, egg homogenates were treated with goat anti-rat IgE as indicated in Fig. 1. The precipitates were dispersed in a buffer containing 0.025 M Tris-HCl (pH 6.8), 2% SDS, 5 mM 2-mercaptoethanol and 10% glycerol and heated for 10 min at 100 °C. Electrophoresis was carried out at 50 V until the sample had passed through the spacer gel, after which 100 V was applied. Gels were stained with Coomassie Brilliant Blue R250 (0.2 g in 100 ml 7% acetic acid in methanol-water 1:1 v/v). After destaining in the solvent, gels were treated with DMSO–PPO, dried and placed in contact with RP Royal X-Omat X-ray film at –70 °C for 2.5 months¹⁴.

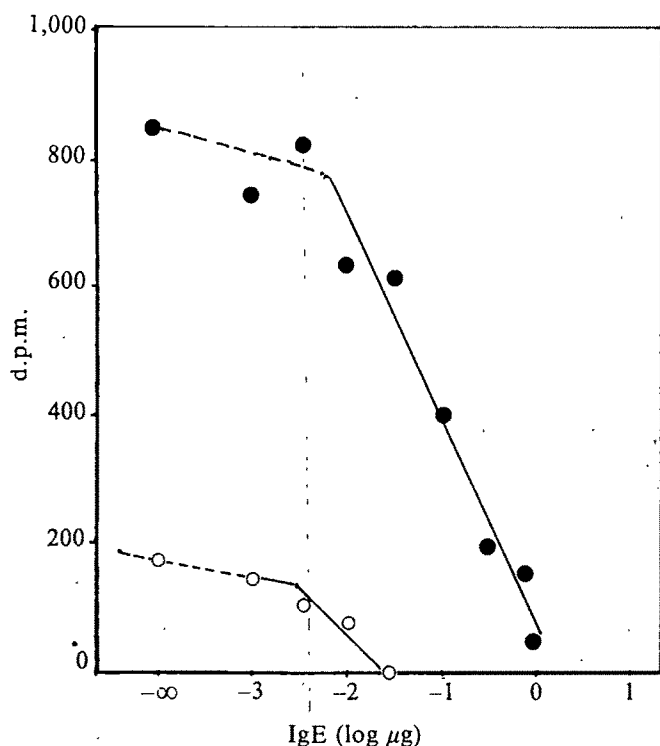


Fig. 3 Dilution experiment by coincubation with purified rat IgE. Before addition of goat anti-rat IgE as described in Fig. 1, the indicated amounts of purified rat IgE (ref. 13) were mixed with the samples. Further procedures were as in Fig. 1 and control values were subtracted. ●, Twelve cells injected per egg, ○, one cell injected per egg.

Electrophoretic analyses of the translation products with myeloma and endogenous RNA are shown in Fig. 2. Most of the radioactivity is found in those parts of the gel where the IgE marker bands are found, whereas in the control experiment, radioactivity was almost exclusively in the buffer front. Further proof of specific translation is given in Fig. 3, which shows that the precipitation of radioactivity with anti-rat IgE is diminished by coincubation with unlabelled purified rat IgE. The results of a similar experiment with the translation products after single cell injection are more variable, but they show the same tendency.

We conclude, therefore, that the *Xenopus* egg translation system makes possible study of the activity of a small number of cells, which can be selected under the microscope by micromanipulation and, as a result, can be very well defined. The minimum number of cells to be used will depend on the amount of messenger per cell, but in our experiments, we could even go as far as a single cell.

Another and, in our view, a very important advantage of our technique, is the fact that no extraction procedures are involved. Part of every RNA extraction is deproteinisation. As a result, masked messengers will contribute their products, whereas they would be inactive *in vivo*. Our method better preserves the native structure of the RNA and, therefore, gives better insight into the actual translational processes in the cell.

We acknowledge cooperation with the Catholic University, Nijmegen. Special thanks are due to Professors H. F. Linskens and J. M. Denucé and to Mr J. R. de Haas. Work in Brussels was supported by the CGER (Brussels), the NIH (USA), and the FRSM (Belgium).

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Received 26 September; accepted 9 November 1977.

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***In vitro* synthesis and characterisation of full- and half-genome length complementary DNA from avian oncoviruses**

RNA tumour viruses carry an RNA-dependent DNA polymerase which can transcribe the viral RNA genome into single-stranded and double-stranded DNA in detergent-disrupted virions^{1,2}. For several years since the discovery of this enzyme, only short DNA fragments of about 100-200 nucleotides long could be produced³. Recently, conditions have been described for the synthesis of possibly full-genome length complementary DNA (cDNA)⁴⁻⁶. The *in vitro* conditions described, however, were not well characterised and did not seem to be very efficient. We report here an efficient synthesis *in vitro* of half- and possibly full-genome length cDNA in avian oncoviruses. These two discrete species of cDNA were synthesised in large quantities. Furthermore, some of the half-genome length cDNA were found to form single-stranded circular molecules with a distinct secondary structure.

The Schmidt-Ruppin strain of Rous sarcoma virus (RSV) of subgroup D (SR-D) was used throughout the experiments. The similar procedures have also been successfully applied to several other strains of avian and murine oncoviruses. Growth of avian oncoviruses in secondary chicken embryo fibroblasts followed the published procedures⁷. The culture media were collected every 12 h. The viruses were purified according to the published methods⁸, and were used immediately for DNA synthesis as described in Fig. 1. We have previously observed that the optimum concentration of Triton X-100 used in the DNA synthesis varied for every virus preparation. It was therefore determined individually for every virus preparation. We also found that the minimum concentration of the viral proteins for efficient synthesis of large cDNA (molecular weight $> 1.5 \times 10^6$) was 1 mg ml^{-1} . At lower concentration, the efficiency of DNA synthesis remained the same, but the molecular weight of DNA products were usually less than 1×10^6 . In contrast to the published report⁹, we found that sodium pyrophosphate inhibited rather than enhanced DNA synthesis (data not shown).

The DNA products synthesised *in vitro* were analysed by using an alkaline sucrose gradient. As can be seen in Fig. 1a, 10-20% of the DNA synthesised had a sedimentation velocity greater than or equal to that of the denatured SV40 DNA marker (molecular weight 1.65×10^6). There were two distinct peaks of DNA products. The one with higher sedimentation velocity (peak I) comigrated with ³²P-35S viral RNA when electrophoresed in a formamide-acrylamide gel (Fig. 1b). This DNA was also shown by electron microscopy to be single-stranded linear DNA

molecules of 9–10 kilobases. About 90% of these DNA molecules could be digested with nuclease S1. After hybridisation with the viral 35S RNA, however, they became completely resistant to nuclease S1 (Table 1). Thus, this DNA peak represented single-stranded cDNA molecules, most of which were near full-genome length. They represented about 5–15% of the total cDNA made. At least 1 μ g cDNA of this species could be synthesised from 5 mg of the purified viruses.

The peak II DNA from the alkaline sucrose gradient had a sedimentation rate roughly equivalent to that of denatured SV40 DNA. This peak consisted of linear DNA molecules of 5–6 kilobases by electron microscopy. As with the full-genome-length cDNA, about 90% of the peak II DNA was sensitive to nuclease S1; after hybridisation with the viral 35S RNA, however, it became completely resistant (Table 1). Thus, they also represented complementary DNA sequences. In three independently prepared lots of cDNA from SR-D, circular single-stranded DNA molecules were observed besides linear DNA molecules (Fig. 2a and b). These circular molecules were very homogenous in size and the contour length of the circle was 5.63 ± 0.38 kilobases, which corresponded to a molecular weight of about 1.8×10^6 . A secondary structure resembling 'rabbit-ears' of distinct length (1.34 ± 0.23 kilobases if traced as completely duplex) and shape were also present in all of the circular DNA molecules (Fig. 2a and b). These circular molecules represented approximately 2% by number of the cDNA from peak II. We believe that these circular molecules were the product of the viral reverse transcription and not cellular contaminants, because no such molecules could be detected in the nucleic acids from the same SR-D virus preparation which had been subjected to the identical purification procedures, including RNase digestion and alkaline sucrose gradient sedimentation. In high formamide hybridisation conditions, these half-genome-size circular DNA could hybridise to the 35S viral RNA genome (for example, Fig. 2c). Unfortunately, such hybrids could only be detected at a very low frequency so that we could only tentatively map the hybrid region at the 5' half of the viral genome, starting at 3.5 kilobases from the 3' end of the genome. The

Table 1 Resistance of the *in vitro* synthesised DNA to nuclease S1

	After hybridisation without RNA	After hybridisation with RNA
Peak I	12%	100%
Peak II	12%	100%
Peak III	39%	85%
Peak IV	29%	79%

1,000 c.p.m. (specific activity $50,000$ c.p.m. μ g⁻¹) of the *in vitro* synthesised ³H-cDNA separated as in Fig. 1a with or without 1 μ g of the 70S viral RNA was used in each experiment. Hybridisation was carried out in 100 μ l of the solution containing 0.9 M NaCl, 0.09 M sodium acetate, 0.05% sodium dodecyl sulphate (SDS) and 1 μ g yeast RNA. The hybridisation mixture was heated at 120 °C for 1 min, chilled immediately and then incubated at 67 °C for 30 min. Afterwards, the hybridisation mixture was cooled gradually over a 12-h period. The nucleic acids were precipitated with 3 volumes of ethanol and then dissolved in 100 μ l of a solution containing 0.03 M sodium acetate buffer, pH 4.5, 0.0018 M ZnCl₂, 0.3 M NaCl and 200 units of nuclease S1 (Miles Laboratories)²⁰. The reaction mixture was incubated at 37 °C for 2 h and the percentage of the ³H-cDNA resistant to nuclease S1 was determined from trichloroacetic acid (TCA)—precipitable counts before and after nuclease S1 digestion.

difficulty in observing this kind of hybrid probably stemmed from the fact that, in the hybridisation conditions used, RNA·DNA hybrid is more stable than DNA·DNA duplex region which is responsible for the circle formation¹⁹. Therefore, most of the hybrids formed between the 35S RNA and the circular half-length cDNA would have become linear.

Thus we have made *in vitro* in high efficiency full- and half-genome-length cDNA from avian oncoviruses. It is interesting to note that the DNA molecules of molecular weight greater than 1.5×10^6 contain only complementary sequences. The shorter DNA fragments, on the contrary, contain both (+) and (−) strands as evidenced by their partial sensitivity to nuclease S1 after hybridisation with the viral RNA (Table 1). The full-length cDNA (peak I) was probably derived from reverse transcription initiated at the primer site to the 5' end of the virion RNA¹¹. The transcription complex may then have jumped to the 3' end of the same or adjacent genome and continued synthesis until

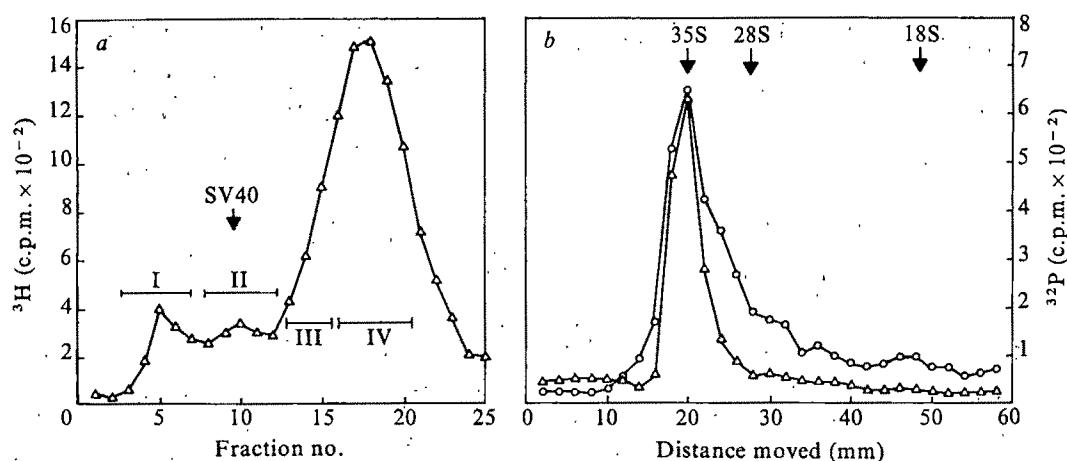


Fig. 1 Characterisation of the ³H-DNA products synthesised *in vitro*. The viruses purified from sucrose gradient⁸ were concentrated by sedimenting on to a 65% sucrose-D₂O cushion containing 0.01 M Tris-HCl, pH 7.4, 0.1 M NaCl and 1 mM EDTA before being used for DNA transcription. The endogenous reverse transcription was carried out in 4–8 ml of the reaction mixture containing 0.1 M Tris HCl, pH 8.0, 30 mM DTT, 3 mM magnesium acetate, 1 mM each of dATP, dCTP, and dGTP, 0.5 mM ³H-TTP (200 mCi mmol⁻¹, New England Nuclear), 1 mg ml⁻¹ of the purified viruses, and 0.02–0.03% of Triton X-100. The optimum Triton concentration was determined beforehand. The reaction mixture was incubated at 40 °C for 18 h. The DNA transcription was stopped by addition of EDTA to a final concentration of 12 mM. The nucleic acids were extracted with phenol three times and then treated with RNase A (50 μ g ml⁻¹) at 37 °C for 1 h. The ³H-DNA products were then further phenolised twice before being precipitated with 3 volumes of ethanol. a, Alkaline sucrose gradient sedimentation of the total DNA products. The ³H-DNA was pelleted by sedimentation at 20,000g for 15 min and then dissolved in 0.3 ml of a solution containing 0.33 M NaOH, 0.5 M NaCl and 0.02 M EDTA. The sedimentation was carried out in a 5–20% sucrose gradient containing the same solution at 40,000 rpm for 11 h at 20 °C in an SW41 rotor; 0.5-ml fractions were collected, and an aliquot of each fraction was counted. b, Fractions 3–6 in a were pooled, neutralised with 1 M acetic acid and then precipitated with 3 volumes of ethanol. The precipitate was dissolved in 40 μ l of phosphate-buffered formamide and electrophoresed in 2.5% formamide-polyacrylamide gel at 80 V for 18 h as described previously¹⁸. The ³²P-35S RNA of SR-D was included as a marker. 28S and 18S RNAs were run in a parallel gel. Δ , ³H c.p.m.; \circ , ³²P c.p.m.

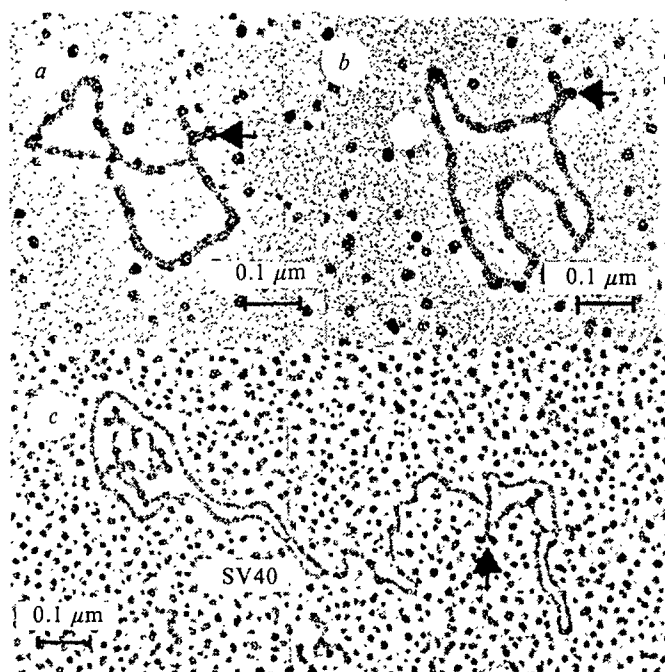


Fig. 2 Electron micrographs of the cDNA synthesised *in vitro*. *a* and *b*, Fractions 8–12 in Fig. 1*a* were pooled and pelleted with ethanol. The DNA was dissolved in 50 μ l H₂O. Aliquots of the DNA were spread from 55% formamide, 0.06 M Tris-HCl (pH 8.5) and 0.006 M EDTA on to a hypophase containing 18% formamide, 0.01 M Tris HCl, pH 8.5, and 0.001 M EDTA. The arrows denote 'rabbit-ear'-like structures. *c*, Heteroduplex between circular cDNA and 35S RNA from SR-D. cDNA 20 μ g ml⁻¹ was hybridised to 35S RNA 10 μ g ml⁻¹ in a solution containing 80% formamide, 0.4 M NaCl, 0.01 M piperazine (pH 6.40) at 46 °C for 30 min. Aliquots of the hybrids were then hybridised to SV40-BrdU (that is, SV40 DNA on to which short poly(BrdU) tails have been added by terminal transferase¹⁹ at room temperature for 20 min in the presence of 0.25 M Tris and 0.025 M EDTA, pH 7.50). The final product was spread with the urea-formamide method described by Kung *et al.*¹⁵.

it reached near the 5' end again¹². Analysis of the DNA fragments of this full-length DNA cleaved by restriction endonucleases supported this interpretation (J. Taylor, A. S. Hsu and M.M.C.L., unpublished). It is not clear, however, where the DNA synthesis terminates. The half-length cDNA might be the result of early termination of transcription. Alternatively, it might have initiated from the same primer near the 5' end and then jumped to the middle of the RNA template. The latter interpretation is consistent with the observations that the early DNA products of reverse transcription contained sequences complementary to those in the middle of the 35S RNA^{13,14}. Preliminary heteroduplex studies suggested that at least part of the half-length cDNA was derived by the latter mechanism. Since the half-length circular cDNA molecules were tentatively mapped at the 5' half of the viral genome, they, too, were probably derived by the latter mechanism.

The formation of the 'rabbit-ear'-like structure in SR-D half-size circular DNA is also curious. This feature is similar to the dimer linkage structure of the RD-114 RNA genome in terms of size and shape¹⁵. We do not know whether it is all double-stranded in this region. Moderate denaturing conditions (55% formamide) did not dissociate this structure. Two possible mechanisms could account for the formation of such structure: (1) If these DNA molecules were the faithful transcripts of the viral genome it would suggest that there are inverted complementary sequences within the viral genome, spaced about 5.6 kilobases. A similar arrangement of genetic sequences has been observed in the genome of herpes simplex virus¹⁶. This arrangement, however, is unlikely since we did not observe such structures in full-length cDNA or 35S viral RNA. (2) If these half-size cDNA molecules represented the products

of aberrant DNA synthesis, for example, a hairpin synthesis at the termination site, then there might be homologous repeated sequences at both the initiation and termination sites for some linear half-size cDNA, which can then form a 'rabbit-ear'-like hybrid region. It has been demonstrated that small double-stranded circular viral DNA of molecular weight 3.4×10^6 could be found in the cells infected with Rous sarcoma viruses (ref. 17 and H. J. Kung, personal communication). The function and fate of these half-size circular viral DNA are unclear. They may be related to the half-size single-stranded circular cDNA of molecular weight 1.8×10^6 we observed in the endogenous reverse transcription *in vitro*.

This work was supported by grants from the NIH and the American Cancer Society. S. H. is a postdoctoral fellow of the Leukemia Society of America. We thank Cynthia Yung and Jean Wang for assistance.

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Received 12 December; accepted 9 December 1977.

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Virus-like particles pathogenic to salivary glands of the tick *Boophilus microplus*

TICK-BORNE viruses are generally transmitted into the host's vascular system in infected salivary secretions¹, and it was to be expected that virus-like particles (VLPs) would be observed during an ultrastructural study of the salivary gland of the cattle tick *Boophilus microplus*. But it is surprising that some of these particles, which are described here, seem to damage the cytoplasm of infected cells, and in extreme cases even to affect the secretory competence of the salivary glands. In view of the economic and medical importance of these arthropods, viruses pathogenic to ticks could have a potential as biological control agents.

Adult female ticks were reared in the laboratory with stalled calves as hosts. VLPs were observed in three different generations collected for 6 months. Salivary glands were prepared as described elsewhere².

Secretory cells within the salivary glands of infected ticks contained large inclusions of regularly aligned VLPs (Fig. 1). Similar particles were not seen in either the synganglion or peripheral nerves from the same ticks. Less heavily infected cells were evident in which individual spherical particles were distributed randomly throughout the cytoplasm. The particles had a diameter of 42–45 nm and consisted of an electron-dense inner core (28–31 nm diameter) surrounded by a less dense outer rim. Some cells from infected tissues were characterised by cytoplasmic

areas which appeared light in intensity when compared with neighbouring regions. These 'plaque' regions were devoid of cytoplasmic organelles and contained both individual VLPs and groups of paracrystalline aggregates (Fig. 2).

In densely infected cells the particles were not observed as paracrystalline arrays but as individual particles which still had the electron-lucent outer rim and the electron-dense inner core (Fig. 3). Occasionally long crystalline filaments were observed in heavily infected cells; these filaments had an electron-dense outer layer and an

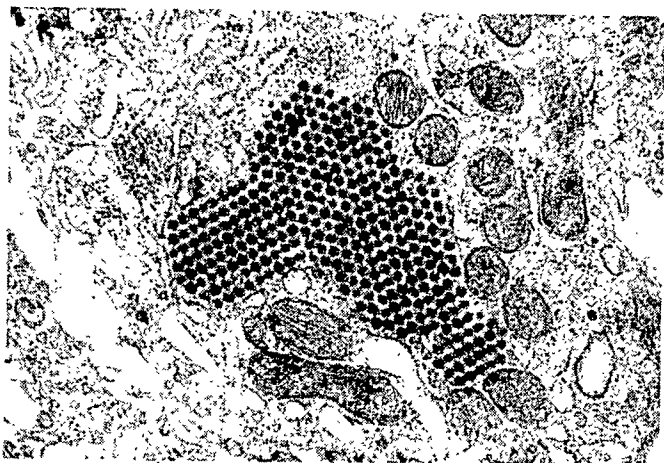


Fig. 1 Group of VLPs in granule-secreting cell from a type II acinus (50,555).

electron-lucent inner core—the reverse arrangement to that seen in the VLPs. It is not yet clear whether the filaments represent a different form of the particle (for example, a self-assembling aggregation of cell synthesised viral protein in excess of that required for virus-particle assembly) or if they are indicative of a different VLP. Similar filaments have been observed in close association with Colorado tick fever virus in cultured hamster kidney cells and in mouse brain cells⁷.



Fig. 2 Plaque region in the cytoplasm of an infected tick (33,000).

Densely infected cells were only seen in salivary glands from abnormally engorged ('plasma') ticks, that is ticks containing large volumes of haemolymph (100–150 μ l, compared with the 'normal' volume of 10 μ l). These 'plasma' ticks have been assumed to have fed on body fluids other than blood. Such an explanation would account for the low haemocrit value of the gut contents⁸ but not for the large haemolymph volumes. It is possible that the dense infections of VLPs observed in the salivary glands of 'plasma' engorged



Fig. 3 Two distinct types of VLP in a granule-secreting cell from a salivary gland of a 'plasma' engorged tick.

ticks reduce the secretory potential of the glands resulting in the accumulation of fluid in the haemocoel.

The presence of the particles described here has been established purely on morphological grounds. Studies directed at the confirmation of the viral nature of the particles and their pathogenic effects are continuing. Some viruses are known to be pathogenic to mites⁹ but so far none has been described in ticks.

I thank Drs J. D. Almeida and K. H. Harrap for helpful comments and the SRC for financial assistance.

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Received 10 October 1977; accepted 3 January 1978.

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Errata

In the letter by R. R. Kay, D. Garrod & R. Tilly, *Nature* 271, 58–60, the title should read 'Requirements for cell differentiation in *Dictyostelium discoideum*'.

In the letter 'Sequences and efficiencies of proposed mRNA terminators' by J. E. McMahon & I. Tinoco, Jr, *Nature* 271, 275, in paragraph 2 line 1, for give read given. In the text figure representing the sequence for two base pairs the dots should be centred between G–C and C–G.

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reviews

Territoriality and adaptation

Jared M. Diamond

Evolutionary Ecology. Edited by Bernard Stonehouse and Christopher Perrins. Pp. vi+310. (Macmillan: London, 1977.) £12.95.

THE late David Lack was a distinguished ornithologist noted for his pioneering evolutionary studies of ecological problems, such as ecological segregation among species, population regulation, territoriality, and reproductive rates. The present volume (not to be confused with the textbook of the same title, by Eric Pianka) consists of a collection of 21 chapters on topics related to Lack's interests, by former students, colleagues, scientific adversaries, and workers whom he influenced at a distance.

The chapters are grouped into four sections. The first five chapters discuss population regulation and territoriality, as illuminated by red grouse (A. Watson), wild rabbits (J. A. Gibb), and great tit (J. R. Krebs), or by controversies that pitted two of the authors (V. C. Wynne-Edwards and D. Chitty) against Lack. Next come four chapters that explore feeding adaptations and ecological segregation in Galapagos seabirds (M. P. Harris), marine Pelicaniformes (J. B. Nelson), seed-eating rodents (M. L. Rosenzweig), and common terns (I. C. T. Nisbet).

The longest section, on breeding adaptations and reproductive rates, consists of eight chapters discussing: arctic geese (I. Newton); great tit (H. N. Kluyver, J. H. van Balen, and A. J. Cavé); cooperative breeding among birds in general (C. H. Fry) and Argentine blackbirds in particular (G. H., C. E., and K. J. Orians); and clutch size in birds (D. F. Owen, C. M. Perrins and R. E. Ricklefs) and in the plant family Compositae (D. A. Levin and B. L. Turner).

The book concludes with four miscellaneous chapters, grouped under the heading of evolutionary adaptations, on systematics of echolocating swiftlets (Lord Medway and J. D. Pye), male group displays in *Chiroxiphia* manakins (D. W. Snow), animal communication systems (A. Zahavi), and big-bang reproduction in yuccas and agaves (W. M. and M. V. Schaffer).

To produce a memorial volume written by 21 different authors or sets

of authors, spanning a wide area of population biology, short enough to sell at a reasonable price, and of high scientific interest is at best a difficult task. The conflicting requirements of these goals weaken the book. Chapters average a frustratingly brief 12 pages in length. This prevents each chapter from giving a major overview of a field or exploring one problem in satisfying detail. The diversity of the content, and the paucity of cross-references between chapters, prevent the volume from developing a 'critical mass' on any single subject except nesting biology. Chapters vary considerably in level of insight. Some good chapters, such as those by Krebs and by Medway and Pye, seem to be so unrelated to the rest of the book that they would have been more effective if they had been published separately as journal articles.

The brevity of chapters is especially unfortunate because population regulation and reproductive rates are complexly determined by multiple factors, the relative importance of which varies with circumstances and with species. In the past, these subjects have often been approached in single-factor, single-species treatments, leading to running controversies that retarded progress. The sections on these two subjects would each have profited from a lengthy chapter synthesising the major factors into an overall scheme, reviewing each factor in detail, and giving examples of species or circumstances where each is important, to place the following chapters in perspective. Overall, I feel that this volume would have been more effective if it had focussed on a narrower span of population biology and consisted of fewer, longer chapters.

These remarks about the book as a whole do not detract from the interest of some chapters taken individually. Three examples must suffice. Watson reviews experiments and observations on the role of territoriality in Scottish red grouse. Between August and November of each year, males establish territories, and females pair with territorial males. The territory provides its owners with exclusive access to food in winter and spring, when food is scarcest and females are forming eggs. Most non-territorial males and unmated

females die of predation or of food scarcity by May. If territorial birds are shot, non-territorial individuals take over the territory, survive well, and breed. Brood size is determined in part by food supply mediated by egg quality formed by females: artificially fertilising the heather results in large broods. Implanting androgen hormone pellets in males makes them more aggressive, and results in their acquiring territories if initially non-territorial or enlarging territories if already territorial.

Newton discusses the extreme set of breeding adaptations that Arctic geese have evolved in response to the short summer. The geese arrive from their southern wintering grounds mated, the females fertilised and ready to lay within a week. Until eggs hatch, the adults survive mainly on fat and protein reserves laid down at wintering and migration areas, and they lose 17–50% of their initial weight. Number of eggs per clutch is greater for early-season clutches or in years with early snow melt. Breeding success varies enormously among years, so that the contribution of young birds to the autumn population may be as low as 1% or over 50%.

Zahavi illustrates the costs incurred by animal communication systems to achieve reliability and minimise cheating. Baby birds beg food from parents by displays that attract the parents' attention but could also attract predators. This risk 'blackmails' the parent into bringing food but inhibits fed nestlings from begging without cause and thereby diverting parents from unfed siblings. Arabian babblers live in groups which jointly defend a territory but of which only one, dominant pair breeds. The dominance hierarchy is maintained not by constant fighting but by a superficially altruistic display: dominant birds offer food to subordinates. That this display represents a dominance manoeuvre rather than altruism becomes clear when a subordinate individual offers food to a dominant one: this constitutes an act of rebellion and immediately provokes a fight. □

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Catastrophe theory

Catastrophe Theory: Selected Papers 1972-1977. By E. C. Zeeman. Pp. 675. (Addison-Wesley: Reading, Massachusetts, 1977.) Hardback \$26.50; paperback \$14.50.

THE strange union of mathematics, philosophy and methodology that has come to be called catastrophe theory sprang entirely from the totally original mind of René Thom. But its widespread popularisation, and its detailed application to the most diverse subjects, is largely the work of Christopher Zeeman. Their writings provide a perfect example of the contrast between the Gallic and Anglo-Saxon intellectual styles—while Thom is grandiose, poetic, elliptic, allusive, impatient with practical detail, sceptical about the possibility that his theory can be put to experimental test, Zeeman is pragmatic, painstaking, pedagogic, tirelessly fashioning ingenious falsifiable models of particular experimental situations, confident that catastrophe theory will form part of the mainstream of applied mathematics.

Thom's great book *Structural Stability and Morphogenesis* has been available since 1972, but Zeeman's most important work has appeared in conference proceedings or journals that are often difficult to obtain. Therefore this publication of a collection of twenty-two of his papers is a very welcome event—all the more so because Zeeman's work has recently come under strong critical attack. Now readers can study the original material and arrive at an informed opinion on the subject.

The mathematics gets steadily more difficult as the book progresses, the culmination being a proof of Thom's theorem (on the classification of singularities of gradient maps—the 'elementary catastrophes') which will be heavy going even for pure mathematicians. The bulk of the book, however, is devoted to applications of the theorem, and it is fortunate that it is possible to understand what it states, and to apply it, without knowing anything of the proof. In restricting himself to applications of the classification theorem (particularly the cusp catastrophe), Zeeman differs from Thom himself, whose speculations often invoke 'generalised catastrophes' the mathematics of which hardly exist as yet.

Space prevents my giving more than a brief indication of the range of Zeeman's applications. At the 'softest' level, they consist of dramatic pictorial encapsulation of the sense of proverbs using the cusp catastrophe; my favourite is "more haste less speed", where haste and skill are conflicting influences

(control parameters) on speed (behaviour variable). Then there are highly developed but still qualitative applications to biology, sociology and psychology. One example is the analysis of the development of the nervous disorder *anorexia nervosa* in terms of the cusp, the hysteresis of which, increasing with the 'splitting factor', models sufferers' sudden alternations between fasting and gorging; a clever invocation of the extra stable sheet of the butterfly catastrophe enables Zeeman to describe the way in which trance therapy can effect a seemingly miraculous cure. In two models (for the onset of prison riots and the impairment of driving skill by alcohol), quantitative data are presented; these are supposed to fit cusp catastrophes, but the points scatter so much that I suspect most readers will share my reaction of being impressed by Zeeman's ingenuity while remaining unconvinced by the evidence.

The 'hardest' applications are in physics. His use of the cusp to describe phase transitions is equivalent to Van der Waals' theory which is well known to fail at the critical point. Of this he

writes: "since density is an averaging device, the shape of the equilibrium surface very near the critical point is slightly distorted". By implication, this dismisses a flourishing area of theoretical physics aimed at understanding the deep reasons for this failure, and such naiveté is likely to cause physicists to think that catastrophe theory can make only trivial contributions in their subject; and that would be a pity. Two of the best chapters in this book, however, also concern classical physics. The first is the elegant and powerful summary of elastic buckling theory. The second is the masterly analysis of the stability of ships, where Zeeman blends the qualitative and the quantitative in an inimitable blend of orthodox naval architecture, topology, group theory and seamen's lore.

My opinion is that for readers interested in applications of catastrophe theory this is the best currently available introduction to the subject.

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Ion-containing polymers

Ion-Containing Polymers: Physical Properties and Structure. By A. Eisenberg and M. King. Pp. 287. (Academic: New York and London, 1977.) \$27.50; £19.55.

THIS second volume in Academic's new series on Polymer Physics presents the first unified treatment of the physical properties and structure of *Ion-Containing Polymers*, which, according to the authors' definition, embraces polymer systems that contain ions and those to which ions can be attached. As one would expect, the book has been thoughtfully planned, no mean feat in a rapidly growing field. It is prefaced by a useful list of symbols; each chapter has a good bibliography; and there are separate author and subject indexes. The format is attractive and the print clear; the vast majority of figures are also excellent but good eyesight is needed to discern detail in a few; CGS units are used.

An introductory chapter describes current knowledge and gives a useful general classification scheme for ion-containing polymers subdivided into two main groups—polymers of low ion content, including Nafion-type systems and ionomers, and polyelectrolytes.

Chapter 2 deals with supra-molecular structure and glass transitions. Theories of and experimental

evidence for multiplet and cluster formation are treated in some detail, although not to mathematical excess. Clustering is seen to be akin to micro-phase separation and to result in many characteristics normally associated with crystalline systems.

Chapters 3 and 4 on the viscoelastic properties of homopolymers and copolymers respectively deal broadly with polyphosphates and silicates; low molecular weight salts in polar polymers and charge transfer complexes; polyelectrolytes; non-crystalline copolymers of high T_g (including perfluorinated ionics, rubber-based ionomers and crystalline copolymers mostly based on ethylene); and polyelectrolyte complexes.

Amongst the properties detailed are stress relaxation, creep compliance, modulus, mechanical loss, specific viscosity, zero shear viscosity, dynamic viscosity, normal stress coefficient and dielectric constant.

The final chapter (5), on configuration-dependant properties, discusses polyelectrolyte dimensions in solution, rubber elasticity, and dilute solution viscosity.

A valuable state-of-the-science book for the research worker in, or wishing to enter, the field of ion-containing polymers, as well as for the professional academic or industrialist.

P. L. Clegg

P. L. Clegg is Head of the Polymer Science Division of the Research Department at ICI Plastics Division, UK.

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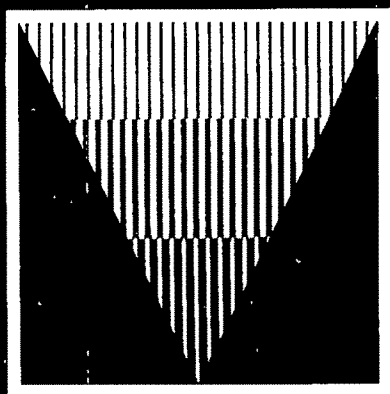
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1703/Jan/78

Notable Victorian naturalist

William Carmichael M'Intosh. By A. E. Gunther (Scottish Academic Press: Edinburgh, 1977.) £5.

THE way of the aspiring academic zoologist in the last century, not least in Scotland, was difficult. It usually involved initial training in medicine. Forbes, Wyville Thompson and Darwin failed to stay the course at Edinburgh. Perhaps W. G. M'Intosh was less fortunate, because graduation qualified him for 22 years' service in lunatic asylums before, in 1882, being appointed to the singularly designated chair of Civil and Natural History at St Andrews. There, however, he remained until 1917, retiring in his 80th year with fourteen years of activity still ahead.

Lifelong interest in marine biology began in childhood rambles on shores around St Andrews, from which his parents and sisters sent parcels of specimens during his years at the Perth District Asylum. He was a naturalist interested in comparative anatomy—highly conventional, holding Darwin in high regard as a naturalist but never really accepting evolution. His name will always be associated with the volumes of his Ray Society Monograph on British Annelids. Taxonomy was a subject to be treated with respect, and frivolities such as the naming, even by Ray Lankester, of a local sipunculid as *Golfingia macintoshi* were to be deplored.

He was involved in early activities

of the Scottish Fishery Board and in the controversies concerning the effects of intensified fishing. He maintained in his *Resources of the Sea* that nothing man did could materially diminish productivity. Pleas for a marine laboratory were answered by a generous donor from Sussex and the Gatty Marine Laboratory was opened in 1896. It was a minor tragedy when the small government grant was stopped and a greater one when his successor, formerly "my junior colleague at Dundee", D'Arcy Thompson, closed it. Already he had offended M'Intosh by participation in the much criticised activities of the International Council for the Exploration of the Sea. Indeed, apart from longevity, no two zoologists could have had less in common.

M'Intosh was a bachelor cared for by his sisters, the youngest of whom, responsible for early illustrations of his annelids, married Albert Gunther, Keeper of Zoology in the British Museum. It is this association that, following his *Century of Zoology*, has led A. E. Gunther to write this welcome account of his great uncle. Based on an unpublished autobiography and a wealth of documented information, it perpetuates the memory of a notable Victorian naturalist and a worthy fighter in what he believed to be right causes. With what happiness would he not view the present flourishing state of the Gatty Laboratory.

C. M. Yonge

Sir Maurice Yonge is Honorary Fellow in Zoology at the University of Edinburgh, Scotland.

Proteins in brain function

Protein Metabolism of the Brain. By A. V. Palladin, Ya. V. Belik and N. M. Polyakova. Pp. 335 (Plenum: New York, 1977.) \$42.

THERE is an active school of Soviet neuroscientists and Professor A. V. Palladin, who died in 1972 at the age of 87, was one of the pioneers. Palladin was particularly interested in the biochemical basis of brain function and this book reflects his interest in the role of proteins in the structure and organisation of the brain.

The book contains a description, usually in chronological sequence, of research into brain protein metabolism, with special reference to the contributions of Soviet scientists. The book was first published in 1972, in its original Russian, the English translation being completed in 1977. Because of this un-

fortunate lapse in time, it has become dated with the latest references being from 1970. Since then, there has been considerable progress in the effects of stress, hormones and drugs on brain protein metabolism. Moreover, our ideas on reutilisation of amino acids and turnover in the brain have undergone revision.

Not surprisingly, much of the basic information described under various subheadings—for example, "Brain Protein Metabolism in Ontogeny" or "Catabolism of Nerve Tissue Proteins"—can be found in more recent reviews dealing separately with these subjects. Nevertheless, the book does have the advantage of bringing together in one volume, the older literature on the varied aspects of brain protein metabolism, together with relevant Soviet literature. Even by today's standards, however, this is an expensive book.

Louis Lim

Louis Lim is Senior Lecturer in Neurochemistry at the Institute of Neurology, London, UK.

Eel biology

The Eel: Biology and Management of Anguillid Eels. By F. W. Tesch. Pp. 434. (Chapman and Hall: London, 1977.) £18.

WHEN I reviewed for these columns the original 1973 German version of this book, I expressed the wish to see it in English. This has now been granted, and Miss Jennifer Greenwood has given us a smooth and impeccable translation.

The sections concerned with endocrinology and sex determination have been brought up to date by Dr Ian W. Henderson. It is a pity that they were not expanded more. For example, two paragraphs on the interrenal organ (adrenocortical analogue) bulldoze over 19 references. Readers would welcome something more satisfying than the enumeration of papers, given that these topics are the growing points of current research on the eel. In fact, 50 out of 98 or so new (post-1972) references given are in the field of physiology. They cover also the feat of inducing sexual maturation of eels in the laboratory.

By contrast, it would seem from the references, that only Dr Tesch himself is currently engaged in the analysis of the eel's migratory behaviour.

The parts on fishery aspects remain the most detailed of the book. The continued interest in the economic value of the eel is shown by the fact that 26 post-1972 papers cited deal with fisheries and fish culture. This is well deserved by an animal that converts into protein for human consumption the insect larvae, worms and snails of rivers and lakes, that is, materials that could not be put to use in other ways. It is interesting to read of the persistent eel re-stocking operations in Eastern Europe and the commercial eel cultures in the Far East.

There have been minor adjustments in other parts of the book too; but no Table nor Figure has been replaced or added on the way. Even the prices of eels are still shown in Table 41 at their 1963 level!

The eel still presents fascinating and instructive problems for investigation. Its physiological versatility, its ambiguous sex determination, the intriguing migration patterns and separation of species, are only some of these. This good English version covering over 1,000 references on the subject, will be of great value to a much wider circle of biologists.

E. M. Pantelouris

E. M. Pantelouris is Reader in Genetics at the University of Strathclyde, UK.

obituary

Maurice Ingram

DR MAURICE INGRAM, former Director of the Agricultural Research Council Meat Research Institute at Langford and Professor of Applied Microbiology at the University of Bristol from 1968 to 1973, died suddenly at his home in Churchill, Avon, on Tuesday 15 November 1977.

Dr Ingram, who was born in Bradford in 1912, was educated at Bradford Grammar School and had a distinguished undergraduate career at Queens' College, Cambridge. After an early interest in botany he became increasingly concerned with food microbiology. His Ph.D. dissertation on *The Effect of Salts in Bacterial Respiration* was presented after joining the Low Temperature Station for Research in Biochemistry and Biophysics where he became Head of the Microbiology Department in 1951.

His early research on bacterial growth and metabolism led to studies of the involvement of pre- and post-slaughter stress factors in the quality and stability of cured products. This work became particularly significant during the second world war when he was engaged in problems of transporting meat and with its salvage after enemy action. In addition he directed research that led to the development of the special rations issued to Allied invading armies and to underground organisations in Europe. He later received the Haakon VII Liberty Medal from the Norwegian Government for his contribution to the partisan attack which destroyed the heavy water plant at Rjukan.

From 1947-49 he studied the bacteriology of whalemeat in an effort to utilise this wasted source of protein. His work on the preservation of concentrated orange juice, which involved travelling to Israel, Italy and Spain, led to the elimination of serious losses. Subsequently he led a team investigating the preservation of foods by ionising radiation. His earlier work again became important in further studies of curing, particularly with reference to food safety and the control of *Clostridium botulinum* by curing salts. He was also active in developing microbial specifications for foods.

In 1963 Dr Ingram was appointed the first Director of the projected Meat Research Institute and as he also became Director of the Low Temperature Station in 1965 he was involved in

the planning and recruitment for the two Institutes which replaced it, the Food Research Institute at Norwich and the Meat Research Institute at Langford. The latter was opened by H.M. the Queen in 1968 and Dr Ingram remained Director until 1973; an honorary degree of Doctor of Veterinary Medicine being conferred on him by the Ludwig-Maximilians University of Munich that year followed by the CBE in 1974.

He retired earlier than necessary to avoid ever-increasing administration which he did not enjoy. Retirement meant that he was able to devote himself more fully to science, and his international reputation ensured that he continued to travel widely, both in a personal capacity and as a consultant for the World Health Organisation. In July 1977 he received the Polish Society of Microbiology Gold Medal of Honour for "most outstanding services to microbiology"—a fitting tribute to a scientist of distinction and a man of great personal achievement.

A man of wide interests and ability he was an accomplished pianist, a keen botanist and, until recently, an enthusiastic and capable cricketer. He spoke French, German and some Norwegian, and read easily in those languages and in Danish. His colleagues will remember him as a man of great integrity, with a mind able to reduce complex questions to simplicity and to answer from a thorough understanding of the basic chemical and physical principles involved. His ability to evaluate arguments logically and to summarise complex discussions concisely made him especially valuable as a chairman at committees.

T. A. Roberts

William Bullerwell

DR WILLIAM BULLERWELL, FRS, FRSE, Deputy Director of the Institute of Geological Sciences, and Chief Geophysicist 1962-1977, died on 25 November aged 61.

After graduating with first class honours in physics in 1937 from Durham University (King's College) he took a further degree in geology, gaining the Lebour Prize for Field Geology, and began research in mining geophysics. This was interrupted by war service during which he

worked on experimental radar, and was mentioned in dispatches.

In the post-war period he joined the Geological Survey of Great Britain, now within the Natural Environment Research Council as part of the Institute of Geological Sciences. Following the pioneer geophysical work for the Survey by J. Phemister and W. F. P. McIntock, he initiated the programme of geophysics that is now a major part of the Institute's work.

In 1950 the Geophysics Division became responsible for collating all gravity data obtained in Great Britain and for its final publication as maps of Bouguer Anomaly: eight quarter-inch to one mile gravity overlay maps were published between 1954 and 1968. During the decade 1955-1965 he supervised the complete regional aeromagnetic survey of the United Kingdom which was published (following interim 1:250,000 scale Diagram Edition sheets) as colour-layered 'ten-mile' aeromagnetic sheets in 1965 and 1972. With his wife, Eileen, whom he married in 1942, and Dr and Mrs James Phemister, during summer vacations from 1953-1956 he carried out the first reconnaissance gravity survey of Scotland.

With the coming of the hydrocarbon search he became deeply involved in the government's research programme of hydrocarbon assessment and search on the continental shelf, especially in advising the Department of Energy. Recently he served on the Advisory Committee of Programme Management for Geothermal Energy of the Commission of European Communities, and from 1970 onwards he was much concerned with offshore prospecting in East Asia as representative for the Ministry of Overseas Development at the Economic and Social Commission for Asia and the Pacific Co-ordinating Committee.

Dr Bullerwell was an exceptionally kindly and generous man who had an encyclopaedic geophysical knowledge that was widely consulted and recognised; and he was elected FRS in 1972 (Council 1974-5) and FRSE in 1973. He served on numerous governmental, university and other professional committees, acting as Treasurer of the Geological Society from 1963-71, and as Chairman of the University of London's Advisory Board on Geophysics.

P. A. Sabine

announcements

Appointments

Lord Todd, O.M., President of the Royal Society has appointed the following Vice Presidents for the year ending 30 November 1977: **Dr B. J. Mason, C.B.**; **Sir Harrie Massey, Professor D. C. Phillips**; **Dr M. G. P. Stoker, C.B.E.**; **Professor P. Allen**; **Dr G. D. H. Bell, C.B.E.**; **Sir Angus Paton, C.M.G.**; and **Sir Peter Swinnerton-Dyer, Bt.**

J. L. van der Post, Director of the British Gas Engineering Station at Killingworth succeeds **Dr R. G. Allen, O.B.E.**, as Director of the Water Research Centre from February.

The following have been elected Emeritus Professors of the University of Bristol: **T. K. Ewer** in Animal Husbandry, **W. A. Gillespie** in Clinical Bacteriology and **R. F. E. W. Peel** in Geography.

Dr Fred Stratton, Director of the Blood Transfusion Service of the North Western Regional Health Authority to Professor (part-time) of human serology at Manchester University.

Awards

The 1977 Papers and Craftsmanship Premiums of the Gordon Radley Fund (Christopher Columbus Prize) awarded by the Post Office to scientists and engineers under 30 for published research work went to **Michel Eve**, **Dr Graham Davies**, **John Davies** and **Robert Walters**.

Michael Heidelberger of New York University, **Elvin A. Kabat** of Columbia and **Henry G. Kunkel** of Rockefeller University have shared the Columbia University's 1977 Louisa Gross Horwitz Prize for their research on the body's disease combating ability and its use in the diagnosis and treatment of disease.

The Paul Martni Prize (20,000 DM) for 1978 will be awarded by the Medizinisch Pharmazeutische Studiengesellschaft e.V., Humboldtstrasse 94, D 6000 Frankfurt. The award is intended to promote the development of scientific methods for the evaluation of clinical, pharmacological and therapeutic fields. Papers, which must be self contained and if published less than 2-yr-old, should be submitted before 30 April 1978.

On the move

Dr Martin Evans from the Department of Anatomy and Embryology, University College London to the Department of Genetics, University of Cambridge.

Dr Norman Shaw from Microbiological Chemistry Research Laboratory, University of Newcastle upon Tyne, to visiting professor in the Department of Chemistry, Washington University.

Dr John Walker from Physics Department, University of Reading and Groupe de Physique des Solides de l'Ecole Normale Supérieure, Université Paris VII, to Quantum Science Corporation, 16 Charles II St, London.

Meetings

3-7 April, **Post-Graduate School on Aspects of Drug Toxicity**, London (R. E. Marshall, Department of Pharmaceutical Sciences, The Pharmaceutical Society of Great Britain, 1 Lambeth High Street, London SE1, UK).

10-14 April, **Symposium on HPLC with application to the Pharmaceutical and Food Industries**, Sunderland (Dr D. Dennis, School of Pharmacy, Sunderland Polytechnic, Chester Road, Tyne and Wear, UK).

12-26 April, **Chromatin Structure and Function**, Erice, Sicily (C. Nicolini, Professor of Biophysics, Temple University, 3223 North Broad Street, Philadelphia, Pennsylvania 19140).

17-20 April, **Institute of Environmental Sciences 24th Annual Technical Meeting**, Texas (B. L. Peterson, Institute of Environmental Sciences, 940 East Northwest Highway, Mount Prospect, Illinois 60056).

19-21 April, **The Origin of Major Invertebrate Groups**, Hull (Dr M. D. Brasier, Department of Geology, The University Hull, UK).

20-26 April, **Remote Sensing of the Environment**, Manila (U-M Extension Service, Conferences and Institutes, 412 Maynard Street, Ann Arbor, Michigan 48109).

25 April, **Fundamental Studies in Keratinisation**, Amsterdam (Professor M. W. Greaves, Institute of Dermatology, Homerton Grove, London E9, UK).

25-26 April, **Some Recent Results in X-ray Astronomy**, London (The Executive Secretary, attn PW, The Royal Society, 6 Carlton House Terrace, London SW1, UK).

6-11 May, **80th Annual Meeting of the American Ceramic Society**, Detroit (The American Ceramic Society Inc., 65 Ceramic Drive, Columbus, Ohio 43214).

9 May, **International Symposium on Crop Protection**, Belgium (Faculteit van de Landbouwwetenschappen, Coupure links 533, B-9000 Ghent, België).

10-12 May, **Biotechnology in Energy Production and Conservation**, Tennessee (Charles D. Scott, Oak Ridge National Laboratory, P.O. Box X, Oak Ridge, Tennessee 37830).

15-17 May, **9th International Symposium on Chromatography and Electrophoresis**, Lake Garda (Dr Alberto Frigerio, Istituto di Ricerche Farmacologiche "Mario Negri", Via Eritrea 62, 20157 Milan, Italy).

15-19 May, **3rd International Conference on Basement Tectonics**, Colorado (Dr Donald L. Baars, Department of Geology, Fort Lewis College, Durango, Colorado 81301).

15-19 May, **1st Australasian Mathematical Convention**, Christchurch (The Convention Secretary, Mathematics Department, University of Canterbury, Christchurch, New Zealand).

Reports and Publications

UK & Ireland—November

Building Britain's Future: Labour's Policy on Construction. Pp. 64. 35p. The EEC and Britain: a Socialist Perspective. Pp. 75. 60p. International Big Business: Labour's Policy on the Multinationals. Pp. 135. 90p. (London: Literature Sales, The Labour Party, Transport House, Smith Square, SW1, 1977.) [211]

Progress in Physical Geography, Vol. 1, No. 1, 1977. Pp. 1-184. Subscription rates: UK, Europe and rest of the world (excl. USA and Canada) £12.50; \$25. USA and Canada \$25. (London: Edward Arnold (Publishers) Ltd., 1977.) [211]

The Edinburgh School of Agriculture. Annual Report, 1976. Pp. 203. (Edinburgh: The Edinburgh School of Agriculture, 1977.) [211]

Occasional Reports of the Royal Observatory, Edinburgh. No. 2: Topics in Extragalactic Astronomy with special reference to the Southern Hemisphere. By Gérard de Vaucouleurs. Pp. vi + 125. (Edinburgh: The Royal Observatory, 1977.) £5. [211]

Physical Impairment: Social Handicap. Pp. 32. (London: Office of Health Economics, 162 Regent Street, W1, 1977.) 70p. [211]

Department of Health and Social Security and the Welsh Office. Advisory Committee on Alcoholism: Report on Prevention. Pp. 14. (London: Department of Health and Social Security, 1977.) [311]

Science Research Council. The Support of Research and Postgraduate Training in Polytechnics—First Report by the Polytechnics Committee to the Science Research Council. Pp. vii + 51. (London: Science Research Council, State House, High Holborn, WC1, 1977.) [311]

Health and Safety Executive. Health and Safety: Mines and Quarries. Pp. v + 58. (London: Health and Safety Executive, 1977. Available from HMSO.) £2, plus postage. [711]

Philosophical Transactions of the Royal Society of London. B: Biological Sciences. Vol. 281, No. 978: Anatomical, Physiological and Biochemical Studies of the Cerebellum from reeler Mutant Mouse. By J. Mariani, F. Crepel, K. Mikoshiba, J. P. Changeux and C. Sotelo. Pp. 1-26 + plates 1-16. UK £5; Overseas £5.15. Vol. 281, No. 979: The Microstructure of the Dentition and Dermal Ornament of Three Dipnoans from the Devonian of Western Australia—a Contribution Towards Dipnoan Interrelations and Moeophogenesis, Growth and Adaptation of the Skeletal Tissues.

- By Moya M. Smith. Pp. 29-72 + plates 1-10. UK £4.90; Overseas £5.05. (London: The Royal Society, 1977.) [711]
- The Safety of the Herbicides 2,4-D and 2,4,5-T. By D. J. Turner. (Forestry Commission Bulletin No. 57.) Pp. 36. (London: HMSO, 1977.) £1.20 net. [711]
- Proctor and Gamble Limited. Annual Report for the year ended 30th June 1977. Pp. 9. Some Basic Beliefs About Marketing. A talk by Edward G. Harness. Pp. 21. (Gosforth, Newcastle upon Tyne: Procter and Gamble Limited, 1977.) [811]
- Freshwater Biological Association. A Key to the British Fresh- and Brackish-water Gastropods, with Notes on their Ecology. By Dr. T. T. Macan. Pp. 46. (Ambleside, Cumbria: Freshwater Biological Association, The Ferry House, Far Sawrey, 1977.) 60p. [911]
- British Nutrition Foundation Annual Report, 1976/1977. Pp. 26. (London: British Nutrition Foundation, 15 Belgrave Square, SW1, 1977.) [1911]
- Radar Design for Determining the Strength of Tropical Cyclones in the Bay of Bengal. By Harold W. Baynton. (A Technical Report.) Pp. 38. (London: Monitoring and Assessment Research Centre, Chelsea College, 459a Fulham Road, SW10, 1977.) £1; \$2. [1911]
- Discarded Containers on a Kent Beach. By Trevor R. Dixon and A. Joy Cooke. (Buckinghamshire College of Higher Education, School of Science and Environmental Studies.) Pp. 13 + tables. (A Report submitted to the Keep Britain Tidy Group.) (Brighton: Keep Britain Tidy Group, National Office, 37 West Street, 1977.) [1911]
- Department of Industry: Committee on Corrosion. Controlling Corrosion. 4: Specifications and Standards. 6: Monitoring. Pp. 12. (London: Committee on Corrosion, Department of Industry, Abell House, John Islip Street, SW1, 1977.) gratis. [1011]
- Health and Safety Executive. Welding and Flame-cutting Using Compressed Gases. (Health and Safety at Work Booklet No. 50.) Pp. 19. (London: HMSO, 1977.) 25p net. [1111]
- Natural Environment Research Council: Institute of Terrestrial Ecology. Ecology of Maplin Sands and the Coastal Zones of Suffolk, Essex and North Kent. By L. A. Boorman and D. S. Ranwell. Pp. 56. (Cambridge: Institute of Terrestrial Ecology, 68 Hills Road, 1977.) £2. [1111]
- J. W. Gregory: Scientist, Explorer, Teacher. A sketch compiled by C. J. Gregory. Pp. 22. (Little Beddow, Chelmsford: M. E. Gregory, Duke's Orchard, Spring Elms Lane, 1977.) [1411]
- National Health Service. Annual Report of the Health Advisory Service to the Secretary of State for Social Services and Secretary of State for Wales for the year 1976. Pp. xii + 94. (London: HMSO, 1977.) £1.75 net. [1411]
- Philosophical Transactions of the Royal Society of London. A: Mathematical and Physical Sciences. Vol. 287, No. 1345: Optimally Scalable Matrices. By T. I. Fenner and G. Loizou. Pp. 307-349. UK £2.60; Overseas £2.70. Vol. 287, No. 1346: A Discussion on the Use of Operational Research and Systems Analysis in Decision Making. Organized by J. F. Coates, FRS, and R. C. Tomlinson. Pp. 351-344. UK £11.20; Overseas £11.60. (London: The Royal Society, 1977.) [1511]
- Science Museum Library. Science Library Bibliographical Series No. 804: 18th Century Nology—Some Writings on the Systematic Classification of Diseases published 1731-1800. Pp. 12. (South Kensington, London: Science Museum Library, 1977.) [1611]
- Ministry of Agriculture, Fisheries and Food: Directorate of Fisheries Research. Fishing Prospects, 1977-1978. Pp. 47. (Lowestoft: Directorate of Fisheries Research, 1977.) [1711]
- Should NATO Keep Chemical Weapons?—A Framework for Considering Policy Alternatives. By J. P. Perry Robinson. (SPRU Occasional Paper Series, No. 4.) Pp. 52. (Brighton: Science Policy Research Unit, University of Sussex, 1977.) [1811]
- The British Library. Research and Development Reports. On-line Information Retrieval: An Introduction and Guide to the British Library's Short-term Experimental Information Network Project. Vol. 1: Experimental Use of Non-medical Information Services. By P. L. Holmes. Pp. viii + 61. (London: The British Library, Research and Development Department, Sheraton House, Great Chapel Street, W1, 1977.) £5. [1811]
- National Radiological Protection Board. NRPB-R55: Fallout in Rainwater and Airborne Dust—Levels in the UK during 1976. By G. J. Hunt, D. J. Elliot and B. M. R. Green. Pp. 16. (Harwell, Didcot, Oxon.: National Radiological Protection Board, 1977. Obtainable from HMSO.) £1. [1811]
- British Gas Corporation. R&D Digest, No. 1, October 1977. Pp. 16. (London: British Gas, 59 Bryanston Street, W1, 1977.) [1811]
- Countryside Commission. Advisory Series No. 2: Interpretive Planning. Pp. 16. (Cheltenham: Countryside Commission, John Dower House, Crescent Place, 1977.) [2111]
- Potato Marketing Board. Annual Report and Accounts 1977. Pp. 44. (London: Potato Marketing Board, 50 Hans Crescent, SW1, 1977.) [2211]
- Medical Textbook Review. Reviewers: Victor Daniels and Steven White. Pp. 63. (Cambridge: Cambridge University School of Clinical Medicine, Addenbrooke's Hospital, Hills Road, 1977.) [2311]
- Wildfowl 28. Edited by G. V. T. Matthews and M. A. Ogilvie. Pp. 176. (Slimbridge, Gloucestershire: The Wildfowl Trust, 1977.) £3.50; \$10. [0000]
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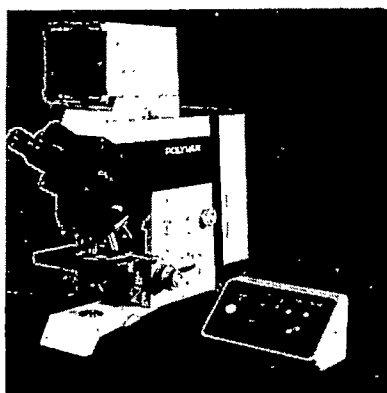
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Blood oxygen equilibrium analyser. TCS Medical Products Division. The Hemox analyser provides an accurate and repeatable blood oxygen association or dissociation curve of normal, abnormal and cord blood. Less than one drop of blood and 20 min of recording time are required to obtain a complete oxygen equilibrium curve of blood. The instrument uses dual-wavelength detection combined with a Clark type oxygen electrode measurement. The resulting output signals are plotted on an x-y recorder.

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Liquid/gel permeation chromatograph. Waters Associates. The Waters Model 150C (150 °C) high-speed, controlled-temperature liquid chromatograph/gel permeation chromatograph provides the ability to analyse materials that dissolve only at elevated temperatures or require higher temperatures to reduce viscosity. This instrument provides rapid results, with 7–10 min separations; it is microprocessor-controlled and may be Factory or Operator Programmed.

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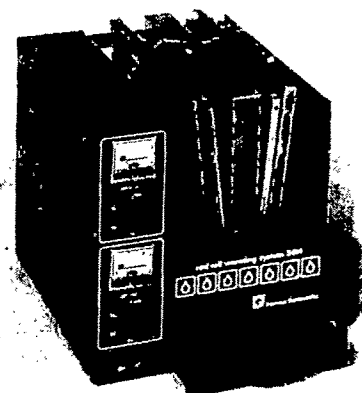
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Acoustic power measuring device. B & K Laboratories. The Type 4205 sound power source consists of two units, the generator, containing all the controls, filters, battery pack, amplifiers and meter, and the sound source, comprising two loudspeakers, associated crossover networks and so on. This combination can be used to produce continuously variable and calibrated acoustic power output up to 100 dB re 1 pW in all directions. Output can be wide band (100–10,000 Hz) or octave band pass filtered. The output can be switched off in less than 30 ms.

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HEAD

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Head, Department of Metallurgy
and Mining Eng.
201 Metallurgy and
Mining Building
University of Illinois at
Urbana-Champaign
Urbana, Illinois 61801
(217)333 1440

1080(A)

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1131(A)

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COMPUTER SERVICES CENTRE
DIRECTOR

The A.N.U. invites applications for the position of Director of the Computer Services Centre. The Centre was established in 1977 following a re-organisation to provide computing and related facilities for both academic and administrative purposes.

In carrying out the work of the Computer Services Centre, the Director will work with a Management Committee for the Centre and will be a member of the committee which deals with University computing policies. In all other respects he will be responsible directly to the Vice-Chancellor.

The Computer Services Centre is equipped with a 2 x 1 Univac 1100/42 which provides remote terminal and batch facilities. Other terminals and peripheral equipment communicate with the central machine through a PDP 11/45. There is also an IBM 360/50 Model H which handles administrative data processing and 11/40 used for development work.

The Director will be responsible for the development of Computer Service and network facilities. On current budget provisions the Centre has a staff of more than 30 personnel organised into programming, operations, technical and director's support teams.

The University is seeking an experienced senior person with professional and tertiary qualifications. Applicants should have management experience in a large organisation and skills in both hardware and software systems. Importance will be attached to capacity to supervise the operation of a centre which is required to provide large scale scientific batch processing and an efficient interactive system involving remote sites.

The Director will be expected to take a prominent part in the formulation of plans for the development of computing services throughout the University. He will be expected to have sufficient experience to negotiate on behalf of the University with computer suppliers and to administer contracts.

A salary of the order of \$A24,000 to \$A26,000 per annum is contemplated.

Reasonable travel and removal expenses will be paid and assistance with accommodation will be provided to the successful applicant if appointed from outside Canberra.

Further information about the position and the Computer Services Centre will be supplied on request to the Registrar, Australian National University, P.O. Box 4, Canberra, A.C.T. 2600 Australia, quoting reference number 78016. The successful applicant will be required to undergo a medical examination. Written applications, quoting reference number 78016, should be forwarded to the Registrar at the above address with whom applications close on **February 28, 1978.**

1280(A)

Research Biologist (Agrochemicals)

May & Baker Ltd., is an international Company manufacturing and marketing a wide range of pharmaceutical, agrochemical and fine chemical products.

A vacancy has arisen in the Bioavailability Research Section at the Company's Research Station at Ongar, Essex, for a biologist to study the effects of physicochemical and environmental factors on the activity of new herbicides, fungicides and plant growth regulators. The work involves laboratory experiments with whole plants grown in controlled conditions, and affords opportunities for teamwork with chemists, and with other biologists including field trials officers.

Applicants should be aged between 23 and 28 years with a first or second class honours degree in botany, plant physiology or plant pathology. Some post-graduate experience with relevant laboratory techniques would be useful.

Conditions of employment include flexible working hours, subsidised meals and a contributory pension scheme with life assurance.

Please telephone 01-592 3060 extension 2506 for an application form or write to



M&B May & Baker

Miss P. Loveday,
Personnel Officer,
(Scientific Staff),
May & Baker Ltd.,
Dagenham, Essex RM10 7XS
quoting reference
number 11/N/1.

1296(A)

UNIVERSITY OF LONDON
INSTITUTE OF
NEUROLOGY
RESEARCH TECHNICIAN

to observe animal behaviour and/or to do biochemical work. Some general duties. Salary £1,824 to £2,910 (plus £450 London Weighting). Curriculum vitae and names of two referees to Professor G. Curzon, Institute of Neurology Labs., 33 Johns Mews, London WC1N 2NS.

1304(A)

UNIVERSITY OF SYDNEY
Australia
PROFESSIONAL OFFICER
SCHOOL OF CHEMISTRY
(Theoretical) (Spectroscopy)

Applications are invited from appropriately qualified persons. Possession of a Ph.D. or equivalent qualification in Physics or Chemistry will be highly regarded. The appointment is initially until December 31, 1978, with good prospects of renewal. Commencing salary in range \$A10,264 to \$A15,703 per annum.

Wide experience in experimental spectroscopy (U.V./visible/I.R.), preferably including laser operation, is essential. The person appointed will take overall responsibility for maintaining and operating existing spectroscopic equipment, and will be expected to be capable of designing further instrumentation. Appointee will be encouraged to participate in the research work of the Department in this area, which is at present concerned primarily with solid-state spectroscopy of mixed-valence systems.

Applications, including details of qualifications and names of two referees, should be sent to the Registrar, University of Sydney, N.S.W., 2006, Australia. Further information may be obtained from Professor N. S. Hush, School of Chemistry in the University.

1279(A)

EXPERIENCED
TRANSLATORS

for English translations of Soviet scientific research journals in chemistry, physics, geology, meteorology, engineering, life sciences and other scientific subjects. English as native language preferred. Send résumé to Box 1260(A).

BAYERO UNIVERSITY
NIGERIA

Applications are invited for the post of PROFESSOR in the DEPARTMENT OF MATHEMATICS.

Applicants should possess a doctorate and have substantial record of published research, together with considerable experience of University teaching at undergraduate and post-graduate levels, including supervision of research programmes. Substantial administrative experience will also be expected and applicants with interests in any branch of Mathematics will be considered. Special consideration will be given to the areas of analysis, algebra and statistics.

Salary scale: N11,268 to N12,420 p.a. (£9,549 to £10,525 p.a. sterling) (£1 sterling=N1.18). There may be supplementation of salary for married appointee of £2,160 p.a. (sterling) (reviewed annually and normally free of all tax) and provision of children's education allowances and holiday visit passages. Single appointee will not attract the supplement. 25% of salary contract addition (partly taxable) is also payable. Family passages; various allowances; superannuation scheme; biennial overseas leave.

Detailed applications (2 copies) including a curriculum vitae and naming 3 referees to be sent by February 28, 1978 to Registrar, Bayero University, PMB 3011, Kano, Nigeria. Applicants resident in U.K. should also send 1 copy to Inter-University Council, 90/91 Tottenham Court Road, London W1P 0DT. Further particulars may be obtained from either address.

1255(A)

San Diego State University announces the establishment of the E.F.F. Copp Chair of Biochemistry. The Search Committee seeks an outstanding scientist whose research interests lie in the areas of diabetes and carbohydrate metabolism with special emphasis on hormone receptors, cell membranes or mechanism of hormone action. Salary is highly competitive and negotiable. Applications should be submitted with curriculum vitae by April 1, 1978 to: Copp Chair Search Committee, Department of Chemistry, San Diego State University, San Diego, CA 92182. An Affirmative Action/Equal Opportunity/Title IX Employer. 1225(A)

UNIVERSITY OF
STRATHCLYDE
PROFESSOR OF
BIOCHEMISTRY

Applications are invited for appointment as Professor and Head of Department of Biochemistry in the School of Biological Sciences following the appointment of Professor P. J. Heald as Dean of the Faculty of Science at the Memorial University, Newfoundland.

Application forms and further particulars (quoting 3/78) may be obtained from the Registrar, University of Strathclyde, Royal College Building, 204 George Street, Glasgow G1 1XW, with whom applications should be lodged by February 28, 1978.

1292(A)

Senior Scientific Officer/ Higher Scientific Officer

£3,254 — £5,778 (plus supplements)

Applications are invited for a pensionable post in the Animal and Crop Husbandry Research Division of the Department of Agriculture located at Hillsborough, Co. Down.

The duties will include investigations into the feeding and management of beef cattle. The successful candidate may also be required to undertake teaching duties in the Faculty of Agriculture and Food Science, Queen's University, Belfast.

Appointment may be at Senior Scientific Officer or Higher Scientific Officer level.

SENIOR SCIENTIFIC OFFICER

Applicants must be over 25 and under 32 years of age on 31 December 1978 and possess a First or Second Class Honours Degree in Agriculture and at least 4 years' appropriate post-graduate experience.

HIGHER SCIENTIFIC OFFICER

Applicants must be under 30 years of age on 31 December 1978 and possess an Honours degree as above and at least 2 years' appropriate post-graduate experience.

Exceptionally applications may be considered from candidates over the age limits who have specialised experience.

SALARY SCALES

Senior Scientific Officer—£4,185—£5,778

Higher Scientific Officer—£3,254—£4,454

In addition to the salary scales quoted pay supplements of between £310.59 and £522.00 per annum will be payable.

Grading and starting salary will be related to qualifications and experience.

Please write or telephone for an application quoting job reference SB 41/78/NA, to the Civil Service Commission, Rosepark House, Upper Newtownards Road, Belfast BT4 3NR (telephone Dundonald 4585 ext 255). Completed forms must be returned to arrive not later than 16th February 1978, 1226(A).



**NORTHERN IRELAND
CIVIL SERVICE**

BIOCHEMISTRY FACULTY POSITION

The Biochemistry Department, University of California, Riverside, invites applications for an Assistant Professor position (tenure track) effective Summer, 1978. Although we have a slight preference for a person with a strong background in physical biochemistry who will initiate research into the structure and function of biologically interesting macromolecules and/or biological membranes, applications from outstanding individuals in other areas of biochemistry will receive serious consideration. The successful candidate will participate in the department's instructional program at the undergraduate and graduate level. Candidates should include in their application a statement of their current and future research objectives and a statement of their teaching experience and areas of competence. Send curriculum vitae, complete bibliography, duplicate copies of three representative publications, and the names and addresses of three references to: BIOCHEMISTRY SEARCH COMMITTEE, Department of Biochemistry, University of California, Riverside, CA 92521. Applications will be accepted until March 10, 1978 or until a suitable candidate is located. The University of California is an Equal Opportunity/Affirmative Action Employer. Minority and women candidates are encouraged to apply. 968(A)

UNIVERSITY HOSPITAL OF SOUTH MANCHESTER

Two research technician positions are available in the laboratories of the University Department of Geriatric Medicine at Withington Hospital within the Geigy Unit for Research in Aging. The work of this Unit is to carry out research into the biology of the aging process and the work on which the appointees will be engaged is concerned with biochemical aspects of this problem.

One person will be required to work with a Postdoctoral Research Assistant and the other to work on other biochemical aspects of aging. Applicants should have O.N.C. or equivalent in biochemistry or a related subject and several years' relevant experience. One post is financed by a M.R.C. grant which terminates at the end of 1979. The other post is supported by Geigy Unit funds and this appointment will be for one year initially, renewable for a further one or two years. Salary up to £2,835 per annum on the Grade 3 technician scale.

Applications including curriculum vitae and the names of two referees should be sent as soon as possible to Dr J. D. Schofield, Geigy Unit, Department of Geriatric Medicine, University Hospital of South Manchester, Nell Lane, Manchester M20 8LR. 1259(A)



**PLYMOUTH
POLYTECHNIC**

Technical Services

RESEARCH TECHNICIAN (MICROBIOLOGY/ BIOCHEMISTRY)

Salary: £2,529 to £2,853
(plus supplement range:
£454.08 to £470.28)

To join research group investigating pathogenicity of bacteria in fish. The successful applicant will work on the purification and mode of action of bacterial toxins.

Applicants should possess H.N.C. or degree in microbiology, biochemistry or related subjects. The project is supported by an S.R.C. grant and is therefore tenable for 15 months in the first instance.

Application forms, to be returned by February 17, 1978, can be obtained with further particulars from the Personnel Officer. 1294(A)

PLYMOUTH POLYTECHNIC DRAKE CIRCUS
PLYMOUTH PL4 8AA

UNIVERSITY OF MELBOURNE

NEW DEVELOPMENT PROGRAMME IN ANTARCTIC STUDIES AND RESOURCES

This new programme is being established as an inter-faculty initiative of which the first phase supports two limited tenure lectureships in the Faculty of Science.

LECTURESHIP (Limited Tenure) in ANTARCTIC GEOPHYSICS DEPARTMENT OF GEOLOGY

Applications are invited for a geophysicist to establish a research and teaching programme concerned with the structure of the Antarctic continent, its continental shelf and its relation to the Southern Ocean and the Australian Plate. Preference will be given to applicants capable of mounting a marine geophysical programme of the Antarctic continental shelf, but others with specific geophysical programmes which could be mounted on the Antarctic continent will also be given full consideration.

LECTURESHIP (Limited Tenure) in BOTANY DEPARTMENT OF BOTANY

A lecturer is required to develop a research programme in some aspects of Antarctic Botany involving, for preference, one or another of the following general areas: marine phytoplankton; marine benthic flora; ecology of terrestrial bryophytes and lichen communities; terrestrial phycology. The appointee will also be required to participate in the teaching of appropriate units at undergraduate and post-graduate levels. Preference will be given to applicants with a Ph.D. or equivalent research degree and post-doctoral research experience.

Both positions will be available for 3 years from date of appointment. SALARY: \$A14,851 to \$A19,551 per annum.

Further information, including details of application procedure, is available from The Registrar, or from the Association of Commonwealth Universities (Apts), 36 Gordon Square, London WC1H 0PF. Applications should be addressed to The Registrar, University of Melbourne, Parkville, Victoria, 3052, Australia. Applications close on March 15, 1978. 1276(A)

UNIVERSITY OF DUNDEE

DEPARTMENT OF BIOLOGICAL SCIENCES

LECTURESHIP

Applications are invited for a Lectureship in the above department from persons with an interest in any field of Plant Biology other than nitrogen metabolism and photosynthetic prokaryotes.

Salary on the scale £3,333 to £6,655 (currently under review) with initial placing dependent on qualifications and experience. Superannuation under U.S.S. Grant towards removal expenses to Dundee.

Applications (6 copies; overseas applicants 1 copy) quoting Reference EST/17/78J and naming three referees should be lodged by February 23, 1978 with The Secretary, The University, Dundee DD1 4HN, from whom further particulars may be obtained. 1188(A)

PORTSMOUTH POLYTECHNIC

RESEARCH FELLOW

A postdoctoral research fellow (S.R.C.) (Male or Female) is required to work on the higher-order structures of Chromatin at a time when studies of the structure of the chromosome are at a rapid stage of development.

The post is in Dr E. M. Bradbury's group and it is expected that a wide range of physical and biochemical techniques will be used including X-ray and Neutron scattering. The successful candidate would probably be an experienced physical Biochemist or a Physicist or Chemist keenly interested in biological problems.

The appointment will be for three years.

Salary: £3,849 to £4,059.

Application forms and further particulars are available from the Staff Officer, Portsmouth Polytechnic, Alexandra House, Museum Road, Portsmouth PO1 2QQ to whom completed applications should be returned by: February 21, 1978. Please quote ref. S44. 1293(A)

UNIVERSITY OF LONDON KING'S COLLEGE

LECTURESHIP IN ANATOMY

Applications are invited for a Lectureship in Anatomy. Preference will be given to candidates interested in teaching Embryology who propose to continue an active programme of research in Development. Applicants should be prepared to assist in the teaching of Topographical Anatomy.

Salary scale £3,355 to £6,655 per annum plus £450 per annum London Allowance. Universities Superannuation Scheme contributions would be payable.

LECTURER/ DEMONSTRATOR IN ANATOMY

A vacancy exists for a full-time Demonstrator in Topographical Anatomy. The post is tenable for twelve months and is suitable for applicants preparing for the Primary F.R.C.S. examination.

Salary scale £3,355 to £6,655 per annum plus £450 per annum London Allowance. Universities Superannuation Scheme contributions would be payable.

Application forms and further particulars for the above posts may be obtained from the Registrar, King's College, Strand, London WC2R 2LS, to whom applications together with the names of two referees should be sent to reach him by Friday, February 24, 1978 quoting reference 191601/N. 1254(A)

BACTERIOLOGIST HEAD OF LABORATORY

An opportunity exists to join a team of microbiologists engaged in chemotherapeutic evaluation of new drugs.

We seek an applicant with experience in bacteriology and perhaps in chemotherapy. Preferred age 28–35 years. His (her) main responsibilities will be the development of new model infections, as well as the performance of efficacy and pharmacokinetic studies.

Our research is founded on an interdisciplinary approach, and successful applicants would have the opportunity to pursue basic research projects in well established research groups studying a wide range of biological problems.

We can offer excellent working conditions in well equipped laboratories. An attractive salary will be paid, and travel expenses from country of origin will be refunded.

Interested scientists are invited to send applications stating age, citizenship, qualifications and experience to Forschungsinstitut, A-1235 Wien, Brunner Strasse 59, Austria. 1307(A)

Senior Research Pharmacologist

A vacancy has arisen at our Welwyn Garden City site for a Senior Research Pharmacologist in the Pharmacology Department.

The Department comprises three sections, cardiovascular, anti-inflammatory and general pharmacology. The successful applicant, male or female, will be required to lead a small project team and contribute to the overall Research effort in the general pharmacology section.

Applications are invited from those with 2 to 3 years' post-doctoral experience who can demonstrate achievements in innovative research.

We offer excellent working conditions in modern and well-equipped laboratories. The salary will be commensurate with experience, additional benefits and facilities include a Christmas Award, contributory pension scheme, free BUPA membership, a subsidised restaurant and, in appropriate cases, assistance towards relocation expenses.

For further information and an application form, please contact the Research Personnel Department (quoting reference RD4) Roche Products Limited, PO Box No 8, Welwyn Garden City, Hertfordshire AL7 3AY, telephone Welwyn Garden 28128. The closing date for applications is 23 February 1978.

1158(A)


ROCHE

IMPERIAL COLLEGE LECTURESHIP IN PLANT PATHOLOGY

Applications are invited for this appointment from Plant Pathologists with special interests in the physiological aspects of diseases caused by micro-organisms. Starting salary up to £5,219 p.a. in the range £3,761 to £6,655 p.a. (under review), according to experience, plus £450 London allowance.

Applications, giving details of experience and the names of two referees, should be sent by March 13, 1978 to Professor R. K. S. Wood, F.R.S., Imperial College, London SW7, from whom further particulars about the appointment may be obtained. 1231(A)

MEDICAL RESEARCH COUNCIL NATIONAL INSTITUTE FOR MEDICAL RESEARCH DIVISION OF VIROLOGY

A scientific staff vacancy will occur in the autumn in a team investigating the fundamental aspects of virus infection using the adenovirus model. The post would be suitable for a researcher with postdoctoral experience in biochemical and/or virological techniques and would be of three years duration with an annual salary, depending on experience, in the range £3,535 to £5,669 p.a. including London Allowance. Superannuation provision.

Applications, giving details of qualifications, experience and the names of two professional referees, should be sent to the Director, National Institute for Medical Research, Mill Hill, London NW7 1AA before February 28 1978. 1243(A)

HOSPITAL FOR TROPICAL DISEASES

4 St Pancras Way, London NW1 2PE

LABORATORY TECHNICIAN

required in the Clinical Nutrition and Metabolism Unit at this Hospital to assist in research on the nutritional effect of surgical trauma and infection. The post would suit state registered persons or others studying for H.N.C. &c. in Biochemistry or Microbiology.

Applications in writing, giving full career details and naming two referees, should be sent to Dr A. M. Tomkins at the Hospital, from whom further particulars are available. 1233(A)

UNIVERSITY OF EDINBURGH

DEPARTMENT OF SURGERY

Head of Department:

Professor G. D. Chisholm

LECTURER—NON-MEDICAL

Applications are invited for the above post which is now vacant. Applicants should be Steroid Biochemists, preferably Ph.D. graduates, with experience in research in reproductive endocrinology. The main research projects are related to hormone measurement and receptor assays in prostatic disease. The salary will be at an appropriate point on the scale £3,333 to £6,655 per annum.

Further particulars may be obtained from the Secretary to the University, University of Edinburgh, Old College, South Bridge, Edinburgh, EH8 9YL, with whom applications (six copies) including curriculum vitae and the names and addresses of two referees, should be lodged not later than February 25, 1978. Please quote Reference 1006. 1270(A)

UNIVERSITY OF IDAHO

Assistant or Associate Professor/Ornamental Horticulturist. University of Idaho. Responsibilities: approximately 80% teaching, 20% research. Ph.D. required. Position available after July 1, 1978. Position open until acceptable candidate is selected. Inquiries should be sent to: Ornamental Horticulture Position, Dept. of Plant & Soil Sciences, University of Idaho, Moscow, Idaho 83843. (Ph. 208/885-6276. A.A./E.O. employer and educational institution. 1148(A)

UNIVERSITY OF NOTTINGHAM

DEPARTMENT OF AGRICULTURE
AND HORTICULTURE

Applications are invited for the post of
RESEARCH ASSISTANT

to work on seed production in amenity grasses at the University of Nottingham School of Agriculture, Sutton Bonington under the direction of Dr P. D. Hebblethwaite, director of the herbage seed research programme.

The project will be financed by Mommersteeg International and will be for a period of three years from October 1, 1978. The successful applicant will be expected to register for a higher degree and should have an honours degree, Upper Second Class or First Class, in either Agriculture, Horticultural Botany, Plant Science or Horticulture. Remuneration will be linked to M.A.F.F. Studentship Maintenance Grants—at present £1,475 per annum.

Applications including a curriculum vitae and the names and addresses of two referees should be sent to A. Whitting, University of Nottingham School of Agriculture, Sutton Bonington, Loughborough, as soon as possible. 1262(A)

THE OPEN UNIVERSITY

Staff Tutor in Biology

NORTHERN REGION

(based in Newcastle upon Tyne)

Applications are invited for a full-time post of Staff Tutor in Biology tenable from 1 April, 1978 or as soon as possible thereafter. The Staff Tutor will be appointed to the Faculty of Science and will be based in the University's Northern Regional Office. The main duties of the Staff Tutor will include liaison with members of the University's central academic staff at Walton Hall and assisting the Regional Director, Northern Region (to whom the Staff Tutor is organisationally responsible), in e.g., the appointment, supervision and training of local part-time tutors. Applications from those with interests in any field of biology are invited but will be particularly welcome from those with interests in neurobiology; mammalian physiology; genetics. Commencing salary will be within the lower part (£3,333 to £4,190) of the Lecturers scale £3,333 to £6,655 per annum, with USS benefits.

Details and application forms are available from The Personnel Manager (STB2), The Open University, P.O. Box No. 75, Walton Hall, Milton Keynes MK7 6AL. Tel. Milton Keynes 63868/9. Closing date for applications 17 February, 1978. 1267(A)

Un laboratoire pharmaceutique
de réputation internationale, désireux de
développer son potentiel de Recherche, engage un

Directeur des Recherches Chimiques et Pharmacologiques

Sous l'autorité du Directeur de la Recherche, il se verra confier la direction et l'animation de notre Centre de Recherches.

Il sera chargé de la coordination entre les chercheurs et le siège, ce qui l'amènera à participer à la définition des orientations de recherche.

jeune Médecin ou Pharmacien

il s'imposera tant par son niveau de compétence scientifique, que par son sens du dialogue et de la communication.

Envoyer dossier de candidature à M. HERVIEUX
10, rue Eugénie - 93240 STAINS
en indiquant la réf. PH sur l'enveloppe.

1224(A)

DEPARTMENT OF METALLURGY AND SCIENCE OF MATERIALS UNIVERSITY OF OXFORD RESEARCH FELLOWS

The Hydration and Development of Strength of Cement

Two posts are available to take part in a project involving research into hydration mechanisms in Portland cement, the effect of modifying additives and the relationship between microstructure and strength. This is an area of Materials Science which would appeal to a person interested in working in an interdisciplinary field involving one or more of the following: electron microscopy, aspects of silicate and colloid chemistry, mechanical property fracture studies.

One post is supported by the S.R.C. and the other by I.C.I. Mond Division and both are tenable for a period of three years. Candidates should possess a Ph.D. degree or equivalent. Salary in the range £3,333 to £5,627 (under review). Applications including a brief curriculum vitae and names and addresses of two referees should be sent to the Administrator, Department of Metallurgy and Science of Materials, Parks Road, Oxford OX1 3PH.

1236(A)

NATURAL ENVIRONMENT RESEARCH COUNCIL INSTITUTE OF GEOLOGICAL SCIENCES GEOPHYSICAL ELECTRONICS TECHNICIANS

The Institute has a vacancy for an Electronics Technician graded Professional and Technology Officer IV in the Applied Geophysics Unit, at BASHLEY ROAD, LONDON NW10. Applicants should know the principles of electronics and have practical experience of electronic instrumentation, together with a familiarity of associated electromechanical devices. A working knowledge of mechanical workshop practice and an ability to use hand tools is desirable. Duties are the inspection, testing, maintenance and repair of an extensive range of geophysical instruments, both in the workshop and the field.

QUALIFICATIONS

- ONC in electrical or electronic engineering or
- an equivalent or higher qualification acceptable to NERC.

All candidates must have served an apprenticeship, or have had equivalent training; in addition they will normally be expected to have at least 3 years' experience. Exceptionally, this latter condition may be waived for candidates with ONC who have completed a 4-year period of apprenticeship or training.

SALARY

£2,425 per annum at age 21, £2,970 at age 27 rising to a maximum of £3,450.

The post attracts Outer London Weighting of £275 per annum. In addition there are supplements to salary of £313.20 per annum and 5% of total earnings, subject to a minimum of £130.50 and a maximum of £208.80 per annum.

ANNUAL LEAVE

20 days per annum, plus 9½ days public and privilege holidays.

Application forms can be obtained from Recruitment Section, Institute of Geological Sciences, Exhibition Road, London SW7 2DE.

Closing date for receipt of completed application forms is 24 February 1978.

Please quote reference PTO/AGU/78/1.

1305(A)

UNIVERSITY OF READING LECTURERSHIP IN SOIL SCIENCE

Applications are invited for a Lecturership in Soil Science.

The person to be appointed will be concerned with teaching and research in the fields of pedology and soil survey, and may be required to take field courses in term and vacation periods. It is hoped to appoint a person with field experience of soil survey work.

The person appointed should take up duties as soon as possible.

Further information may be obtained from the Registrar (Room 214, Whiteknights House), The University, Whiteknights, Reading RG6 2AH, by whom applications should be received not later than March 31, 1978.

1266(A)

THE CHINESE UNIVERSITY OF HONG KONG

invites applications for the post of Technician (with prospects of promotion to Senior Technician) in Electron Microscopy in the Department of Biology. Requirement: a university degree or a diploma/certificate from a polytechnic in biology with experience in Electron Microscopy. Preference will be given to candidates with a strong electronics background.

Salary range: £2,700 to £4,860 p.a. approx. for technician; £5,100 to £5,860 p.a. approx. for senior technician depending on qualifications and experience. Also superannuation (15% University, 5% employee), medical benefits, annual and sick leave. Appointee is expected to assume duty on June 1, 1978.

Further information and application forms are obtainable from the Personnel Section, C.U.H.K., Shatin, N.T., Hong Kong. Applications, marked "APPLICATION" on cover, should be returned before March 15, 1978.

1237(A)

COMMONWEALTH AGRICULTURAL BUREAUX Vacancy for SCIENTIFIC INFORMATION OFFICER

at the

COMMONWEALTH FORESTRY BUREAU Commonwealth Forestry Institute

South Parks Road,
Oxford OX1 3RD

Duties of post: The scrutiny of technical literature, selection and preparation of abstracts in many subjects related to forestry or forest products (this may involve visits to outside libraries), indexing, preparing for the press and dealing with technical enquiries.

Flexible working hours are based on 37 hours per week. Applicants wishing to work 18½ hours per week, with appropriate adjustments of salary, etc., will be considered.

Qualifications: Applicants should be graduates in forestry or in a biological science or in a relevant branch of physical or engineering science. A good reading knowledge of a modern language, preferably Russian, German, French or Spanish, is required.

Salary: In the scales £2,149 to £3,527 to £4,454 plus cost-of-living supplement and a compensatory allowance (taxable but not superannuable) of 41% to offset personal contribution to F.S.S.U. Starting salary according to qualifications, experience and age. Promotion to higher scales on merit.

Application forms and full particulars from the Executive Director, Commonwealth Agricultural Bureaux, Farnham House, Farnham Royal, Slough SL2 3BN.

Closing date for applications: February 27, 1978.

1251(A)

TECHNICIAN required in Department of Chemical Pathology for general day to day duties required for running of a section of the Department and to assist with research project involving bacterial cultures and enzyme assays. Salary according to experience and qualifications within scale £2,982/4,259 p.a. plus £354 p.a. London Allowance. Apply with full details and names of two referees to, The Secretary, St Mary's Hospital Medical School, Paddington, London W2 1PG quoting Ref. T/CP/N.

1253(A)

UNIVERSITY OF OXFORD AGRICULTURAL RESEARCH COUNCIL

Unit of Muscle Mechanisms and

Insect Physiology

DEPARTMENT OF ZOOLOGY RESEARCH ASSISTANT

Applications are invited for a post, financed by the Muscular Dystrophy Association of America, to work on the composition and structure of muscle Z discs. Experience in protein biochemistry and/or electron microscopy essential. Candidates should have a good first degree; the post would also be suitable for a postdoctoral worker. The vacancy is available immediately and is for one year with the possibility of renewal. Salary scale Research Support 1B (£2,904 to £4,811 p.a.) or 1A (£3,333 to £5,627 p.a.) with U.S.S. benefits.

Applications with Curriculum vitae and names of two referees to Professor J. W. S. Pringle, A.R.C., Unit, Department of Zoology, South Parks Road, Oxford, before February 20,

1288(A)

FACULTY POSITION— PLANT BIOCHEMIST

Applications are requested for an appointment as ASSISTANT PROFESSOR OF BIOCHEMISTRY in the tenure track at the University of California, Riverside, effective Summer 1978. Applicants should have demonstrated ability in research in plant biochemistry and the capability to develop a vigorous research program in this area. This individual will be expected to teach a course in plant biochemistry at the upper division/graduate level and participate in other teaching activities of the department. Candidates should send curriculum vitae, a statement of their current and future research interests and ask three scientists familiar with their work to send us letters of recommendation. Applications will be accepted until March 10, 1978 or until a suitable candidate is located. Address correspondence to: SEARCH COMMITTEE, PLANT BIOCHEMIST, Department of Biochemistry, University of California, Riverside, CA 92521. The University of California is an Equal Opportunity/Affirmative Action Employer. Minority and women candidates are encouraged to apply.

969(A)

THE UNIVERSITY OF LEEDS DEPARTMENT OF BIOCHEMISTRY

Applications are invited for the post of:

LECTURER

in the above Department. The person appointed will participate in teaching biochemistry to science and to medical students. Facilities and opportunities for research are excellent. The appointment will date from October 1, 1978. Salary on the lecturer scale £3,333 to £6,655.

Application forms and further particulars may be obtained from the Registrar, The University, Leeds LS2 9JT, quoting reference number 83/10/D. Closing date for applications March 10, 1978.

1248(A)

UNIVERSITY OF
MELBOURNE
LECTURESHIP IN
SOIL SCIENCE
SCHOOL OF AGRICULTURE AND
FORESTRY

Applications are invited for the above-mentioned position, commencing October 1, 1978. The appointee will be required to lecture in the Soil Science courses given in the School of Agriculture and Forestry and to participate in the associated practical classes.

The appointee will be encouraged to develop his own research interests and to participate in postgraduate training programmes. Preference will be given to those applicants interested in biological aspects of soil science or chemical aspects of soil fertility.

QUALIFICATIONS: Ph.D. or equivalent, with some postdoctoral experience.

SALARY: \$A14,851 to \$A19,551 per annum.

Further information, including details of application procedures and conditions of appointment, is available from The Registrar, University of Melbourne, Parkville, 3052, Victoria, Australia, or from the Association of Commonwealth Universities (Apts), 36 Gordon Square, London WC1H 0PF. Applications should be addressed to The Registrar and close on March 15, 1978.

1277(A)

UNIVERSITY OF ZAMBIA

Applications are invited for the post of
SENIOR LECTURER/
LECTURER

in the
GEOLOGY DEPARTMENT
SCHOOL OF MINES

Applicants should possess a higher degree and teaching or research experience in the following fields: Applied Geochemistry, Economic Geology and Structural Geology.

Salary scales: Senior Lecturer K6,324 to K7,200 p.a.; Lecturer K4,248 to K6,108 p.a. (£1 sterling = K1.42). A local supplement of salary is likely to be provided; this is paid by mining companies. The British Government, may supplement salaries at a level which will allow the overall income of appointees to equate with other staff members whose salaries are supplemented by the British Government. Family passages; baggage allowance; superannuation and medical aid schemes; regular overseas leave.

Detailed applications (2 copies) together with curriculum vitae and naming 3 referees to be sent to Registrar, University of Zambia, P.O. Box 2379, Lusaka, Zambia, by February 28, 1978.

Applicants resident in U.K. should send one copy of application to Inter-University Council, 90/91 Tottenham Court Road, London W1P 0DT. Further particulars may be obtained from either address. 1303(A)

THE HANNAH
RESEARCH INSTITUTE
SCIENTIFIC OFFICER

in the
BIOCHEMISTRY DEPARTMENT

A vacancy exists in the Biochemistry Department for a Scientific Officer to assist with work on the metabolism by rumen micro-organisms of aromatic compounds present in forage plants and the effects that these metabolic processes have on the utilisation of dietary polysaccharides. Candidates should have a degree, H.N.C. or equivalent, in Chemistry or Biochemistry.

Salary according to qualifications and experience in scale £2,592 to £4,032 including current pay supplements. Non-contributory superannuation.

Further particulars may be obtained from The Secretary, The Hannah Research Institute, Ayr KA6 5HL, to whom applications, with full curriculum vitae and the names and addresses of two referees should be sent by: February 27, 1978. 1290(A)

THAMES POLYTECHNIC
SCHOOL OF
MATERIALS SCIENCE AND
PHYSICS
X-RAY AND NUCLEAR
LABORATORY
TECHNICIAN GRADE 5

Applications are invited for a technician for the X-ray and nuclear laboratories who will be required to operate X-ray equipment, handle radioactive sources and be responsible for keeping exposure records. Candidates should have a recognised apprenticeship or H.N.D./H.N.C. qualification and at least eight years' relevant experience.

Salary scale: £3,027 to £3,483 including London weighting plus personal allowance (i.e. 5% of gross pay; min. £2.50, max. £4.00).

Further particulars and form of application may be obtained from the Staffing Officer, Thames Polytechnic, Wellington Street, London SE18 6PF, to whom completed applications should be returned by February 14, 1978. 1242(A)

THE UNIVERSITY AT
ALBANY
BEHAVIORAL ECOLOGY
FULL PROFESSOR

The Department of Biological Sciences is seeking a senior scientist of stature in the area of Behavioral Ecology for an appointment beginning fall 1978. The department is interested in applicants who currently maintain an active research program and who have demonstrated substantial scientific achievements. The appointee will be expected to provide direction and leadership for the ecology/animal behavior program which currently consists of six faculty. The department has approximately 70 graduate students in M.S. and Ph.D. programs.

Please send curriculum vitae and three references to Kenneth P. Able, Chairman of Search Committee, Department of Biological Sciences, SUNY at Albany, 1400 Washington Avenue, Albany, NY 12222.

The State University of New York at Albany is an Equal Opportunity/Affirmative Action Employer.

1263(A)

UNIVERSITY OF CRETE

The Physics Department of the University of Crete will admit its first freshman class in the 1978-79 academic year at the Sciences Campus near Knossos. During the 1978-79 year the organisation of both the undergraduate and graduate curricula will be completed and the administrative structure of the Department will be established. Faculty positions are open at all levels. Applications for visiting as well as regular appointments are solicited. The visiting appointments will be made in the Spring of 1978 and some regular ones will be made during the 1978-79 year. Send all required documents to Professor D. Katakis, 71 Solonos Street, Athens 143. Send a copy of your curriculum vitae and reference letters to Prof. G. C. Alexandrakis, Chairman, Department of Physics, University of Miami, Coral Gables, Florida 33124 (Acting Chairman, Physics Department, University of Crete). 1265(A)

PALYNOLOGIST

Applications are invited for a Post-doctoral Fellowship in palynology. The Fellow will collaborate on a project investigating Holocene environmental changes in the Lake Manitoba Basin. The appointment may be made as early as May 1978 and will be for 1-2 years, subject to funding approval. Send resume and names of three referees to Dr J. T. Teller, Department of Earth Sciences, University of Manitoba, Winnipeg, Manitoba, Canada, R2T 2N2. 1261(A)

The Daresbury Laboratory has a vacancy at Senior Scientific Officer level for a

Solid State Theoretician

The fields of interest of the Theory Group at Daresbury are solid state, atomic and molecular, and nuclear.

At the Laboratory a purpose-built X-ray and ultraviolet source and a major nuclear structure facility are under construction. An IBM 370/165 computer is installed at the Laboratory, and a computational science group has recently been established working mainly in the quantum chemistry area.

The appointment would be a permanent one at Senior Scientific Officer level made according to age and experience in the range £4,185 to £5,778. In addition there are pay supplements of (a) £312.00 per annum and (b) 5% of total salary subject to a minimum of £130.50 per annum and a maximum of £208.80 per annum. There is a non-contributory superannuation scheme and a generous leave allowance.

The successful applicant, of either sex, will be expected to take a strong interest in synchrotron radiation experiments which are being planned on the new source. Applicants should possess a Ph.D degree and several years' research experience in solid state theory.

Closing date: 1 April 1978

Please write enclosing curriculum vitae, the addresses of two referees and quoting reference number DL/611/T to:

The Personnel Officer

1227(A)

DARESBURY LABORATORY
Science Research Council
Daresbury, Warrington WA4 4AD

UNIVERSITY OF
QUEENSLAND
DEPARTMENT OF
PHYSIOLOGY
PHYSIOLOGIST
(TECHNOLOGIST B)

A graduate is required who is experienced in or has an interest in the field of endocrinology or fetal physiology. Preference may be given to a person experienced in the development of radio immunoassays for steroid hormones or prostaglandins.

The duties would be to establish the above techniques, supervise the training of technical staff and to collaborate in the Departmental research programs in these areas.

Further information may be obtained from Professor G. D. Thorburn in the University.

Salary: \$A382.10 to \$A570.20 per fortnight.

Applications, quoting reference No. 00178 and providing the names and addresses of 2 or 3 referees, should be forwarded to the Staff Officer, University of Queensland, St Lucia, 4078, Qld. Australia. 1278(A)

UNIVERSITY COLLEGE
HOSPITAL MEDICAL
SCHOOL
TECHNICIAN

to work in the field of leukaemogenesis. Work will entail attempts to define factors leading to host resistance and mode of inheritance of leukaemia in mice, and will involve fine techniques such as surgical manipulation of early mouse embryos as well as general handling of mice. Experience in mouse handling and record keeping is essential, whilst a working knowledge of basic virological and immunological techniques would be an advantage. Although part of a small team, the successful applicant must be prepared for the responsibility of carrying out some of his or her duties alone.

Written applications in first instance to Professor D. V. I. Fairweather, (N) Department of Obstetrics and Gynaecology, University College Hospital Medical School, 88-96 Chenies Mews, London WC1E 6HX, from whom further information may be obtained. 1301(A)

QUEENSLAND AGRICULTURAL COLLEGE

A MULTI-VOCATIONAL
COLLEGE OF ADVANCED EDUCATION
LAWES (GATTON)
QUEENSLAND, AUSTRALIA

DIRECTOR

The Council of the Queensland Agricultural College invites inquiries and applications for the position of Director of the College, which will become vacant on the retirement of the present Director in November, 1978.

The College, situated 96 kilometres West of Brisbane on the Brisbane/Toowoomba Highway, is one of ten autonomous colleges of Advanced Education in Queensland. It has a current enrolment of 750 students and offers a wide range of courses in the Rural Technology, Rural Management, Food Technology, Food Service Management and Hospitality Management fields, at the degree, diploma, associate diploma and certificate levels.

The Director is Head and Principal Academic and Administrative Officer of the College. The successful applicant will be an imaginative and purposeful leader. He or she will have had extensive relevant academic, practical and administrative experience.

The salary and other conditions compare favourably with similar senior academic posts throughout Australia.

Conditions of appointment and further details of this position and of the Queensland Agricultural College are available from The Chairman of the Council Mr E. P. S. Roberts, to whom inquiries and applications should be addressed at Queensland Agricultural College, Lawes, Queensland, Australia, 4345.

Applications close on May 1, 1978.

1250(A)

**UNIVERSITY OF
WARWICK
RESEARCH TECHNICIAN
THE DEPARTMENT OF
BIOLOGICAL SCIENCES**

has a vacancy in the microbiology research group for a contract technician to work on the isolation and purification of enzymes from methane oxidising bacteria. Applications are welcomed from graduates and others with appropriate qualifications and experience in biochemistry and/or microbiology.

Salary will be either on the Technician Grade 2B Scale, £2,529 to £2,880 p.a. or Grade 3 at £2,688 to £3,069 p.a.

Apply by letter giving full details to the Academic Registrar, University of Warwick, Coventry CV4 7AL, quoting Ref. No. 26/2T/78, by February 9, 1978. 1295(A)

**UNIVERSITY OF
LIVERPOOL
DEPARTMENT OF
BIOCHEMISTRY**

Applications are invited from biochemists for the postdoctoral post of Senior Research Assistant.

To work on the properties and regulation of synthesis of the ethanolamine ammonia-lyase of *Escherichia coli*. Enzyme formation appears to uniquely require the concerted action of two inducers. Familiarity with the concepts and techniques of bacterial genetics as well as enzymology would be advantageous. The post, tenable for a period of up to two years from a date to be arranged offers a salary in the range of £3,333 to £3,547 per annum with superannuation benefits.

Applications, together with the names of two referees, should be received not later than February 28, 1978, by the Registrar, The University, P.O. Box 147, Liverpool L69 3BX, from whom further particulars may be obtained. Quote Ref: RV/660/N. 1244(A)

**THAMES POLYTECHNIC
SCHOOL OF
BIOLOGICAL SCIENCES
RESEARCH ASSISTANT**

Applications are invited from good honours graduates in a biological discipline for the post of Research Assistant in the Division of Physiology and Cell Biology, School of Biological Sciences. The successful applicant will be required to work on the ultra-structural and cytochemical aspects of synapse formation in insects, under the supervision of Dr D. J. Beadle, and will be registered with the C.N.A.A. for a higher degree.

Salary: £2,778 to £2,940 inclusive. Further particulars and form of application may be obtained from the Staffing Officer, Thames Polytechnic, Wellington Street, London SE18 6PF, to whom completed applications should be returned by February 14, 1978. 1240(A)

**UNIVERSITY OF READING
DEPARTMENT OF PHYSIOLOGY
AND BIOCHEMISTRY
POSTDOCTORAL
CARBOHYDRATE CHEMIST**

immediately required for up to two years for the synthesis of sugar derivatives and metabolites for W.H.O. sponsored research on the control of male fertility. Experience with radioisotopes an advantage. The Chemist will work with Professor G. M. H. Waites in the Department of Physiology and Biochemistry and Dr R. Khan in the Philip Lyle Memorial Research Laboratory, University of Reading.

Salary up to £3,761 per annum (under review). U.S.S. superannuation. Apply quoting Ref. M.N.02A, with curriculum vitae and two academic referees to Assistant Bursar (Personnel), University of Reading, Whiteknights, Reading RG6 2AH. 1274(A)

**THE LONDON HOSPITAL
MEDICAL COLLEGE
(University of London)
RESEARCH ASSISTANT
BIOCHEMIST**

Applications are invited for the appointment of Biochemist with good honours degree and also preferably a higher qualification, to join a small team investigating the physical biochemistry of articular cartilage and the intervertebral disc. This post is financed by a grant from the M.R.C.

Further enquiries to Dr A. Maroudas, tel: 01-247 5454, ext. 421.

Initial salary within the range £3,333 to £4,190 (under review) plus 100 London Allowance per annum.

Applications (two copies quoting RA/Biochem.2/78) together with the name of one referee to the Secretary, The London Hospital Medical College, Turner Street, London E1 2AD, within 14 days. 1281(A)

**UNIVERSITY OF
EAST ANGLIA, NORWICH
FRESHWATER BIOLOGIST**

A post is available immediately as Research Associate to help investigate the limnology of the Norfolk Broads. The project will include water chemistry and invertebrate analyses as well as experimental work; field work will be carried out the year around and the ability to co-operate in a team is essential. The appointment will be for one year at a salary of £2,904 per annum, in the first instance but may be renewable for up to three years for a suitable person. Candidates should have a good honours degree appropriate to freshwater biology and should be able to drive. Applications should be sent as soon as possible to Dr Brian Moss, School of Environmental Sciences, University of East Anglia, Norwich NR4 7TJ, and should quote two referees who are readily contactable. 1297(A)

**UNIVERSITY OF
NEWCASTLE upon TYNE
CHAIR OF ANATOMY**

Applications are invited for the Chair of Anatomy which will become vacant on April 1, 1978, through the resignation of Professor J. J. T. Owen. The Chair will carry with it the Headship of the Department of Anatomy for an initial period of five years which will be renewable. The candidate selected will be required to take up appointment as soon after April 1 as possible.

Salary will be in accordance with the Professorial scale, with membership of the appropriate superannuation scheme.

Further particulars may be obtained from the Registrar of the University, 6 Kensington Terrace, Newcastle upon Tyne NE1 7RU, with whom applications (15 copies), giving the names of not more than three referees, must be lodged not later than March 6, 1978. (Applicants from outside the British Isles may submit one copy only.) 1282(A)

**LONDON SCHOOL OF
HYGIENE AND
TROPICAL MEDICINE
(University of London)
Keppel Street, WC1E 7HT
DEPARTMENT OF
ENTOMOLOGY
TECHNICIAN**

required to assist for up to three years in a W.H.O.-funded programme on in vitro culture of human filariae using insect cell lines. Candidates should have H.N.C. in a relevant discipline. Applications in writing, giving full career details and naming two referees, should be sent to Secretary (A1). 1300(A)

**LONDON SCHOOL OF
HYGIENE AND
TROPICAL MEDICINE
(University of London)
Keppel Street, WC1E 7HT
DEPARTMENT OF
MEDICAL MICROBIOLOGY
BACTERIOLOGY UNIT**

Applications are invited from good honours graduates in the biological sciences, medicine or veterinary medicine for a post of Research Fellow, tenable up to three years, for research into microbiological pathogens in wildlife, with special reference to *Mycobacterium bovis*. Opportunities exist for the successful applicant to prepare for a higher degree. Salary, depending on experience, etc., in the range £3,377 to £4,640 inclusive. Applications, in writing, giving full career details and naming two referees, should be sent to the Secretary (A1). 1299(A)

**Central Birmingham Health
District
THE CHILDREN'S
HOSPITAL
TECHNICIAN or
BIOLOGICAL SCIENCE
GRADUATE**

required for a research post. The successful applicant will be concerned with a leukaemia and non-Hodgkins Lymphoma study. Previous experience in immunology helpful but not essential. Appointment for one year in the first instance.

Salary according to experience and qualifications.

For further details and an application form please contact Dr F. G. H. Hill, Consultant Haematologist, Haematology Department, The Children's Hospital, Ladywood Middleway, Birmingham B16 8ET. Telephone: 021-454 4851. 1246(A)

ASSOCIATESHIPS

**UNIVERSITY OF
CALGARY
GRADUATE
ASSISTANTSHIPS
(Teaching and Research).**

are available for students proceeding to the M.Sc. and Ph.D. degrees in all branches of CHEMISTRY and BIO-CHEMISTRY. The assistantships are valued currently from \$6,000 to \$7,000 per annum and carry remission of program fees and travel allowance. Positions are available beginning September 1978.

For further information write to: Department of Chemistry, Chairman of Graduate Studies, UNIVERSITY OF CALGARY, Calgary, Alberta, Canada T2N 1N4. Please indicate field of interest 1252(P)

**UNIVERSITY OF
MANCHESTER**

A Postgraduate Research Assistantship is immediately available for an APPLIED

MATHEMATICIAN

The successful candidate would work in the Department of Obstetrics and Gynaecology, in close collaboration with the Department of Mathematics, on problems in applied mathematics arising from the medical sciences. He or she may be expected to register for a higher degree. Appointment for one year in the first instance.

Salary £2,904 to £3,761 p.a. Superannuable.

Curriculum vitae and names of three referees should be sent to Dr B. S. H. Rarity, Department of Mathematics, The University, Manchester M13 9PL, as soon as possible. 1284(P)

STUDENTSHIPS

**AGRICULTURAL
RESEARCH COUNCIL
FOOD RESEARCH INSTITUTE
A.R.C. RESEARCH STUDENTSHIP**

Applications are invited from students for an Agricultural Research Council Studentship tenable from October 1, 1978 for up to three years at the A.R.C. Food Research Institute with a view to reading for the degree of Ph.D. at the University of East Anglia. The award is in the Microbiology Division where the student will work with Dr B. M. Lund on alkali-tolerant microorganisms from food wastes.

Details of the award are available from: The Secretary, A.R.C. Food Research Institute, Colney Lane, Norwich, Norfolk NR4 7UA, to whom application should be made in writing by February 28, 1978. 1230(F)

**THE WEST OF SCOTLAND
AGRICULTURAL COLLEGE
RESEARCH STUDENTSHIP**

Applications are invited for a Research Studentship in the Botany Department, to study the use of herbicides in the improvement of bracken-infested land. The award is tenable for up to three years, commencing in October, 1978, and the holder will be required to register for a higher degree.

Further details and application forms may be obtained from the Secretary, The West of Scotland Agricultural College, Auchincruive, Ayr KA6 5HW. 1249(F)

**S.R.C. "C.A.S.E." STUDENTSHIP IN
BIOMEDICAL ENGINEERING**

Applications are invited from Graduates or those hoping to graduate this year for a "C.A.S.E." award tenable now under the supervision of Dr G. W. Hastings in association with the Orthopaedic Department, North Staffordshire Royal Infirmary for work on development of an artificial tendon prosthesis for use in injured hands. Candidates should have a degree in Polymer Science or Technology, Materials Science, Fibre Science or Chemistry.

Apply to: Dr G. W. Hastings, Bio-Medical Engineering Unit, Medical Institute, Hartshill, Stoke-on-Trent,

enclosing curriculum vitae and addresses of two referees. 1256(F)

STUDENTSHIPS

continued from previous page

THE HANNAH
RESEARCH INSTITUTEfor Studies Relating to the Production
and Utilisation of Milk
AGRICULTURALRESEARCH COUNCIL
RESEARCH STUDENTSHIP

Applications are invited for an A.R.C. Research Studentship tenable for up to three years for research leading to a Ph.D. degree. Candidates should have, or expect to obtain in 1978, a 1st or Upper 2nd Class Honours Degree in an appropriate field. The successful candidate will be expected to carry out a research project in one of the following areas:

1. Hormonal control of amino acid metabolism;
2. Transport and metabolism of lipids;
3. Factors affecting the heat stability of milk;
4. Energy and protein metabolism in the cow.

Applications giving *curriculum vitae* and the names and addresses of two referees should be forwarded to:

The Secretary,
The Hannah Research Institute,
Ayr KA6 5HL,
by April 14, 1978, from whom further particulars can be obtained. 1289(F)

A.R.C. ANIMAL
BREEDING RESEARCH
ORGANISATION
POSTGRADUATE
STUDENTSHIP

Applicants are invited for an A.R.C. Research Studentship tenable in Edinburgh for three years from October, 1978.

The successful student will study the use of ultrasonic measurements on the live animal for the prediction of carcass quality with a view to obtaining a higher degree during the three years.

Candidates should have graduated, or be about to graduate in agriculture, agricultural science, zoology, physiology or a closely related field.

Awards include a tax-free allowance, payment of fees and other expenses. Full details and application forms are available from the Secretary, A.R.C. Animal Breeding Research Organisation, West Mains Road, Edinburgh EH9 3JQ, Scotland.

Applications should be made by March 4, 1978. 1268(F)

FELLOWSHIPS

UNIVERSITY COLLEGE OF
NORTH WALES, BANGORDEPARTMENT OF
BIOCHEMISTRY AND
SOIL SCIENCEPOSTDOCTORAL
RESEARCH FELLOWSHIP

Applications are invited for an S.R.C. Postdoctoral Fellowship (in collaboration with Professor W. Charles Evans) to undertake biochemical studies on the Anaerobic Metabolism of Aromatic Compounds by Micro-organisms.

The appointment is from March 1, 1978 or as soon as possible thereafter, and will be for a period of 22 months, ending on December 31, 1979.

Starting salary according to age and experience on Scale £3,333 to £5,627 per annum.

Applications (two copies), quoting reference 78/29, together with the names and addresses of two referees should be sent to the Assistant Registrar (Personnel), University College of North Wales, Bangor, Gwynedd LL57 2DG, from whom further details may be obtained. 1298(E)

RESEARCH FELLOWSHIP

IMPERIAL

CANCER RESEARCH
FUND LABORATORIES

Mill Hill, NW7

A postdoctoral Fellow is required to join a group working on the genetic and biochemical analysis of certain heat activated loci in *Drosophila melanogaster*, using cloned DNA fragments to investigate their organisation and expression.

Appointment will be for two years in the first instance with possible extension for a third year. Salary with entry according to qualifications and experience within range £4,649 to £5,669.

Further information from Dr D. Ish-Horowicz (01-959 3236).

Applications with *curriculum vitae* and names of two referees, should be sent to The Secretary, Imperial Cancer Research Fund, Lincoln's Inn Fields, London WC2A 3PX, by February 28, 1978. 1208(E)

UNIVERSITY OF
SALFORDDEPARTMENT OF
CHEMISTRY AND
APPLIED CHEMISTRYPOSTDOCTORAL
RESEARCH FELLOWSHIP

Applications are invited for a Postdoctoral Research Fellow to work in association with Professor R. B. Cundall and Dr R. H. Bisby on a project sponsored by the M.R.C. entitled "The role of enzymic repair and chemical radiosensitisers in the yield of strand breaks in irradiated DNA."

Experience in radiation biochemistry desirable, but not essential.

The appointment is tenable for up to three years, commencing as soon as possible.

Salary within the range £3,333 to £3,761 p.a. with U.S.S.

Application forms obtainable from the Registrar, University of Salford, Salford M5 4WT (Telephone 061-736 5843) extension 215, to whom completed applications should be returned not later than Monday, February 27, 1978 quoting reference CH/217/N. 1291(E)

RESEARCH
FELLOWSHIP

A POSTDOCTORAL FELLOW required as soon as possible for an appointment of two years with possible extension to three years.

The work will be on the mechanisms by which viruses convert cells from a normal to malignant state, with emphasis on leukaemia-inducing viruses.

Salary with entry according to qualifications and experience within range £4,649 to £5,669.

Further information from Dr J. Wyke (tel: 242 0200, ext. 267/270).

Applications with *curriculum vitae* and names of two referees, should be sent to:

The Secretary,
Imperial Cancer Research Fund,
Lincoln's Inn Fields,
London WC2A 3PX,
by March 17, 1978, quoting Ref. 61/78. 1209(E)

UNIVERSITY OF OXFORD

UNIVERSITY LECTURERSHIP IN
PHYSICAL CHEMISTRY

Applications are invited for a University Lectureship in Physical Chemistry, at a stipend according to age on the scale (under review) £3,333 at age 24 or under to £7,087 at age 42 or over, with superannuation. The post may be held in conjunction with a tutorial fellowship at University College, in which connection attention is drawn to the separate advertisement of this fellowship.

Further particulars of both posts may be obtained from Professor J. S. Rowlinson, Physical Chemistry Laboratory, South Parks Road, Oxford OX1 3QZ, to whom applications (six copies, except in the case of applicants outside Europe, who need send only one) with the names of three referees, should be sent by February 28, 1978.

FELLOWSHIP IN PHYSICAL CHEMISTRY,
UNIVERSITY COLLEGE

University College proposes to elect a Fellow and Praelector in Physical Chemistry. The post is open to men and women. The fellowship must be held in conjunction with the University Lectureship currently being advertised. Further particulars may be obtained from Professor J. S. Rowlinson, Physical Chemistry Laboratory, South Parks Road, Oxford OX1 3QZ, to whom applications should be sent by February 28, 1978. 1247(E)

UNIVERSITY OF
BIRMINGHAMRESEARCH FELLOWSHIP
IN GEOCHEMISTRY

Applications are invited for the above postdoctoral Fellowship in connection with a N.E.R.C. supported research programme to study the volcanic and plutonic rocks of the Scotia Arc and Antarctic Peninsula. Experience in igneous geochemistry is essential. The post is tenable for 22 months from April 1, 1978. Salary in the range £3,333 to £5,627. Maximum starting salary £4,190 (plus superannuation).

Applications (three copies), naming three referees, should be sent to the Assistant Registrar, Science and Engineering, University of Birmingham, Birmingham B15 2TT, by February 20, 1978. Please quote ref: NG2. 1269(E)

SITUATIONS WANTED

B.Sc., M.Sc. Pharmacologist, minor chemistry, experienced in the analysis of drugs in blood by polarography and glc, pharmacokinetics and statistical analysis of data with computer. Box 1257(B).

CONFERENCES

4th EUROPEAN
MEETING ON
BACTERIAL
TRANSFORMATION
AND TRANSFECTION
University of York,
York, U.K.

August 29–September 1, 1978

The following topics will be included in the programme:

DNA binding and uptake in
Transformation
Recombination in
Transformation
Plasmid Transformation
Transformation in Genetic
Engineering
Transformation Related to
Medicine
Transfection

Speakers will include: S. Lacks; M. Fox; G. Venema; A. M. Sicard; S. N. Cohen; G. Humphreys; F. E. Young; S. Erlich; P. F. Sparling; W. Wackernagel; T. Trautner.

Further details from: Secretariat, 4th European Meeting on Bacterial Transformation and Transfection, 142 Oxford Road, Cowley, Oxford OX4 2DZ, U.K. 1285(C)

LECTURES

N.A.T.O. ADVANCED STUDY INSTITUTE
1978 SUMMER SCHOOL on

"Synchrotron Radiation applied to Biophysical, Biochemical and Biomedical Research"

will be held in Frascati (Rome—Italy) from August 20 to September 1, 1978.

The subject will be covered by an international Faculty of outstanding lecturers. Lectures will be given in English in the format of a School to an audience of Scientists willing to use Synchrotron Radiation in their own field of Research. Persons willing to receive more information and application forms should write to:

Professor I. F. Quercia—Laboratori Nazionali
P.O. Box 56—FRASCATI
(Rome—Italy)
tel. 06/940262-06/941041

1234(K)

COURSES & SYMPOSIUMS

SCHOLARSHIPS

UNIVERSITY OF SALFORD
in association with the
PERKIN DIVISION OF THE CHEMICAL SOCIETY
International Symposium on
CHEMISTRY AND BIOCHEMISTRY OF PROSTANOIDS
will be held at

THE UNIVERSITY OF SALFORD
July 11-14, 1978

Symposium Chairmen

Dr S. M. Roberts (Salford) and Dr F. Scheinmann (Salford)
Invited Speakers will include:

- Dr W. Bartmann (Hoechst Aktiengesellschaft, Frankfurt)
Dr M. P. L. Caton (May & Baker Limited, U.K.)
Professor D. A. Van Dorp (Unilever Research, The Netherlands)
Professor J. Fried (University of Chicago, U.S.A.)
Professor P. A. Grieco (University of Pittsburgh, U.S.A.)
Professor E. W. Horton (University of Edinburgh, U.K.)
Dr R. L. Jones (University of Edinburgh, U.K.)
Dr S. Moncada (Wellcome Research Laboratories, U.K.)
Dr J. Muchowski (Syntex Research, U.S.A.)
Dr R. F. Newton (Allen & Hanburys Research, U.K.)
Dr C. Pace-Asciak (Hospital for Sick Children, Toronto)
Dr R. Pappo (Searle Laboratories, U.S.A.)
Dr J. E. Pike (Upjohn Company, U.S.A.)
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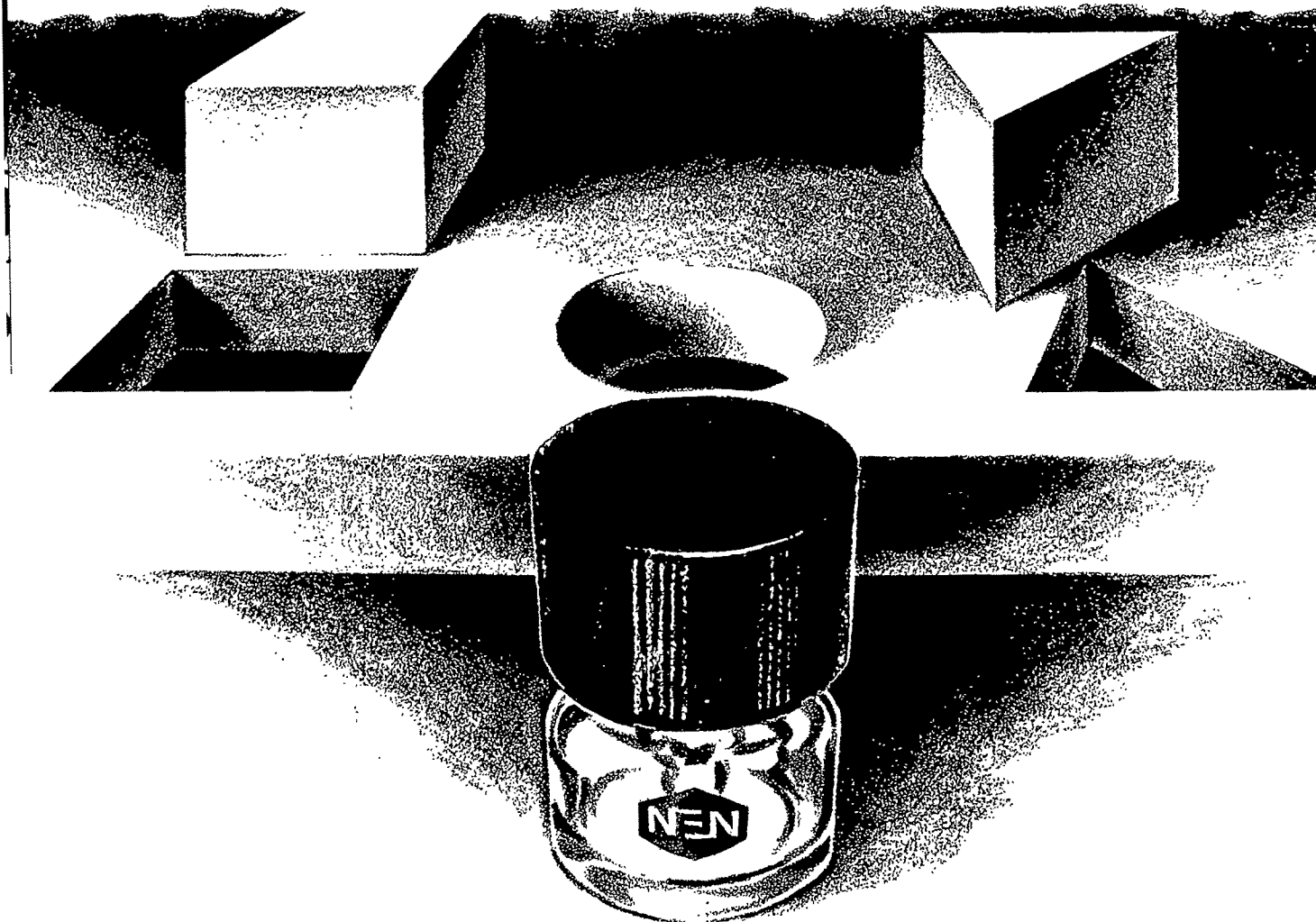


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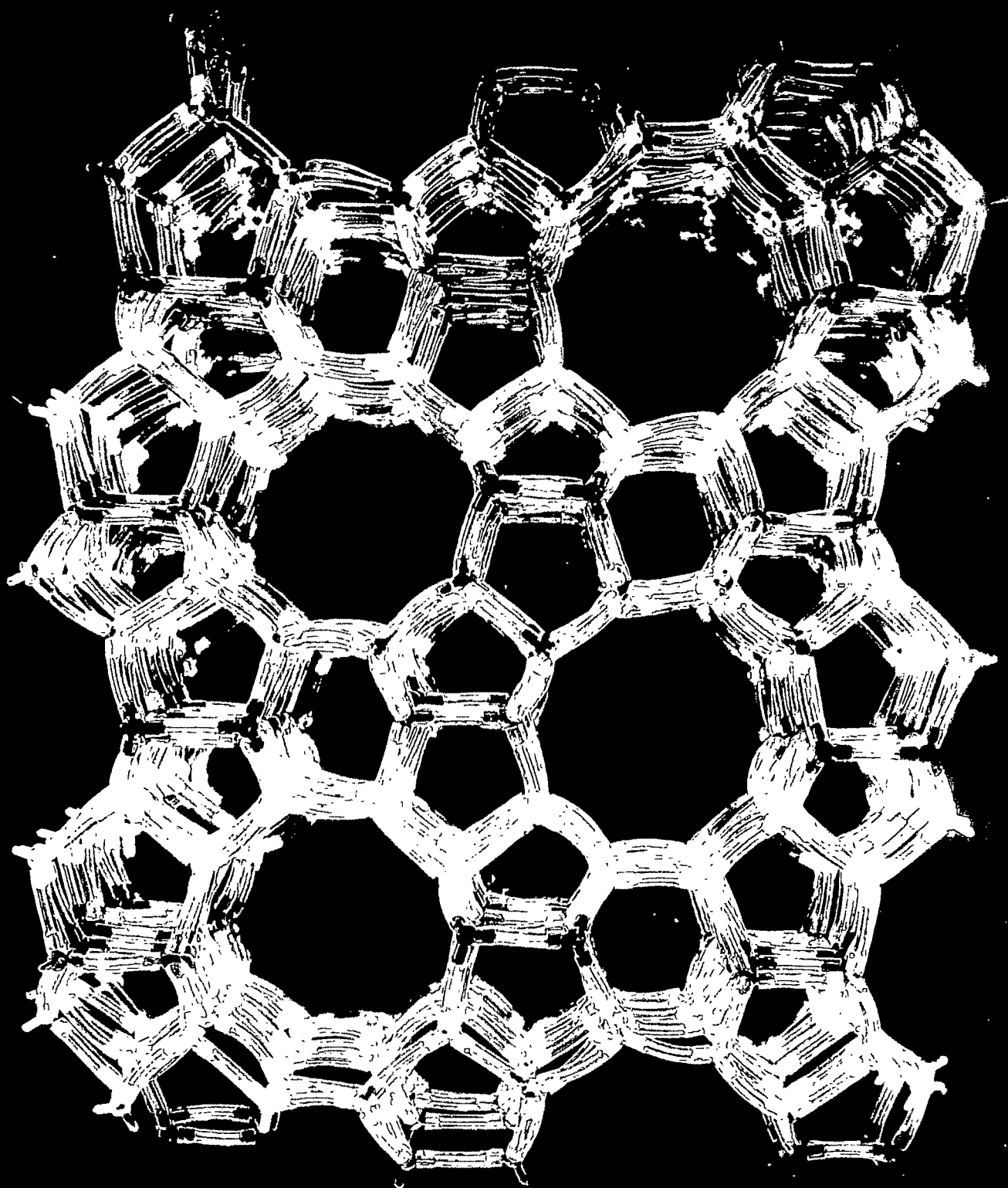
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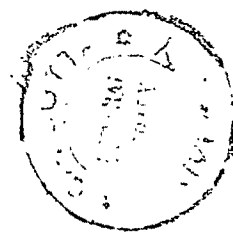
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ISSN 0028-0836

Registered as a newspaper at the
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silicon atoms at the tetrahedral stars
are linked by plastic spaghetti to illus-
trate the framework topology.
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9 February 1978

Little comfort for university teachers

Two and a half years ago the present Labour government of the UK introduced the first stage of a pay policy which is, of course, still in operation. In its first year the policy laid down that the maximum salary increase for anyone would be £312, and that those already being paid more than £8,500 per annum should get no increase at all. Succeeding stages have been equally tight-fisted. There can be little doubt that the policy has been effective in reducing the level of inflation from around the 25% per annum mark to the present 10%. But there have been obvious losers—the levelling nature of the policy has ensured substantial declines in real income for those paid above average, and the necessity for an arbitrary date of imposition in 1975 ensured that pay anomalies unresolved at that time remain unresolved. Nor in the present gradual thaw in incomes policy is there much comfort for those who live under the discipline of public sector cash limits or who are unable to negotiate deals on productivity. Into every one of these losing categories fall university teachers.

The 1975 clampdown occurred at a time when university teachers had gone to arbitration over their claim for the adjustment of parities with polytechnic staff and over a cost of living increase; they were left stranded. Everyone agrees that they have had a raw deal, and the salary scales speak for themselves: the lecturer scale starts at £3,333 per annum and professors can start as low as £8,100. At the low end of the pay scales, lecturers are widely able to claim social benefits that are intended for the poorer paid members of the community. More senior university teachers have seen their salary decline in real terms by up to 20% in the past few years, and by more when one includes 'fiscal drag'—the failure of tax rates and thresholds to move with the cost of living.

Even though there has been much hand-wringing by the government about the plight of university staff, it is not yet at all clear what the present negotiations between the Association of University Teachers (AUT) and the employers will yield. AUT, notably without industrial muscle, has said it is prepared to live with a rise that is consistent with the present 10% guidelines, but only with a firm timetable set for the elimination of the present anomalies. The union's expectation is that the anomalies would be removed by October of this year, and it has been handed a very fine precedent for such hopes by the firemen, who accepted an offer close to the guidelines only on the promise that they

would be well looked after in future years (assuming no change in the political complexion of the government). This form of agreement is not the sort of thing the government wants to repeat, however, and it has made the somewhat lame excuse that the firemen were unique because 'human life was at stake'. So university lecturers (who have actually increased their 'productivity' with significant increases in the student: staff ratio in the past few years, but who save no lives) are not held out much comfort.

The worrying thing in all this is not just the poor salary levels; it is a growing feeling that the Labour government is moving towards a perceptibly different style for universities without being entirely open about its intentions. A few weeks ago, the Commons debated the situation in the universities, including, of course, the pay problem, but much else besides. Two Labour MPs independently spoke about the need for greater public control of what goes on in universities, one going so far as to say that there was "a growing feeling in the country, particularly amongst younger Socialist politicians, that there should be a greater degree of public accountability for university funding". The Minister of State, Department of Education and Science, with special responsibility for higher education, Mr Gordon Oakes, did not exactly dissociate himself from such ideas, mentioning that the Labour Party had a discussion document on them, and going on to say that he hoped that in ten years "the university . . . will contain a 50-50 mixture—50 per cent coming from schools and 50 per cent who could be of any age".

From a minister, even to an almost empty House, this looks rather heavy stuff. Many, of course, will agree in general with his remarks about responsiveness but point out that a lot of universities, particularly those recently converted from colleges of advanced technology, scamper around quite a lot in an effort to meet the nation's wishes. But is he serious about a half-and-half university by 1988? Certainly there are demographic changes which will start to reduce the numbers of school-leavers after 1981, and certainly there is much scope for more continuing education. These most recent remarks, however, cannot but be unsettling at a time when university staff morale is not high. If ideas are indeed that advanced, the government should publish them, together with documentation, and give individual members of the university community a chance to have a reasoned discussion and give a sensible response. □

Saccharin: the risks and benefits

Bernard L. Cohen of the University of Pittsburgh argues that the decision on whether or not to ban saccharin should be taken in the light of risk-benefit analysis. The risk of cancer may be outweighed by the risks of obesity.

SCIENTIFIC decision-making in matters of public concern should be made on the basis of risk-benefit analysis; but in the controversy over the banning of saccharin there has been little evidence that this has been done. That controversy involves decision making by the general public, so it is important that the public understand the risks in some quantitative fashion.

Since the average person does not readily accept numbers, the best substitute is to compare the risks of saccharin with other risks with which members of the public are familiar. It is my purpose here to give both a risk-benefit analysis and to compare saccharin risks with other risks familiar in our daily lives. All calculations will assume a linear relationship between dose and effects as is customary in estimates of this type.

In the recent Canadian study of bladder-cancer patients (*Lancet*, 17 September 1977, page 578), a possible link was established between that disease and the use of saccharin such that if the United States population (2×10^8) were to ingest one diet soft drink each day throughout their lives, there would be an extra 1,200 bladder cancers per year. This implies a risk of 1,200 cancers per 7.3×10^{10} drinks, or one cancer per 6×10^7 drinks. There is ordinarily a time delay of 10 to 50 years between ingestion of a carcinogen and development of a cancer, so an average case would result in no more than a 20 year loss of life expectancy; thus, based on the results of the Canadian study, an average diet drink would reduce life expectancy by 20 yr/ 6×10^7 , or about 9 s.

To give a perspective on this number, smoking a single cigarette reduces life expectancy by 12 min (B. L. Cohen *American Scientist* 64, 550; 1977) so a diet soft drink is about 80 times less dangerous than a cigarette. From the above result (or from the original finding) it is straightforward to calculate that one diet drink per day throughout life causes a reduction in life expectancy, ΔL , of 2 d; or

$$\Delta L = 2 \text{ d} \left(\frac{\text{diet drinks}}{\text{d}} \right) \quad (1)$$

The benefits of diet soft-drinks result from their use in weight control by reducing calorific intake. Being overweight is well known to reduce life expectancy. In a rather old study, Pauling (*Proc. Natl. Acad. Sci.* 44, 619; 1958) analysed the data on this in a 1952 report of the Metropolitan Life Insurance Co. to obtain best fits to both a linear and quadratic relationship between loss of life expectancy, L , and overweight ($W - W_0$), where W is the weight and W_0 is the optimal weight. These were,

$$L = 17 \text{ yr} [(W - W_0)/W_0] \quad (2)$$

$$L = 36 \text{ yr} [(W - W_0)/W_0]^2 \quad (3)$$

Differentiating (3) and assuming that an average saccharin user would be at least 10% overweight and would weigh perhaps 160 lb gives

$$\Delta L = 0.05 \text{ yr/lb} \cdot \Delta W \quad (4)$$

Applying the same assumptions to (2) gives

$$\Delta L = 0.11 \text{ yr/lb} \cdot \Delta W \quad (5)$$

independent of the percentage overweight.

There is more recent data on this subject from the same source (*Metropolitan Life Insurance Co. Statistical Bulletin* 10; 1960). To quote typical figures, for a 45-year-old male with 150 lb optimal weight, weighing 170 lb reduces life expectancy by

1.5 years and weighing 200 lb reduces it by 4 years. This gives an approximately linear relationship with

$$\begin{aligned} \Delta L &= 0.08 \text{ yr/lb} \cdot \Delta W \\ &= 29 \text{ d/lb} \cdot \Delta W \end{aligned} \quad (6)$$

Since this is intermediate between (4) and (5) and is based on better data, I shall use (6).

An average person's body weight is related to his average daily calorie intake at about 1 lb per 14 calories per day. Multiplying this by (6) gives a change in life expectancy

$$\Delta L = 2 \text{ d/calories-per-d-intake} \quad (7)$$

From a comparison between (1) and (7) we see that diet soft drinks give a net benefit if one such drink reduces calorific intake by more than 1 calorie.

There seems to be no firm evidence on the amount by which diet drinks reduce calorific intake (or body weight). A non-diet drink has about 100 calories, so if all other things were unchanged, substituting diet for non-diet drinks increases life expectancy by 100 times more than the cancer risk reduces it. This is perhaps an extreme assumption, but it would be very difficult to prove that it over-estimates the reduction in calorie intake from diet drinks by a factor of 100. Unless this is done, there is no proof that the risk from diet drinks is greater than their benefits, and in fact it seems most likely that the opposite is the case.

If, on the other hand, we ignore the foregoing discussion and assume that there are no benefits from use of saccharin, it becomes important to understand the risks. We have already noted that ingesting a diet drink is 80 times less dangerous than smoking a cigarette, and we discuss here some other comparisons.

Perhaps the simplest risks to quantify are those due to automotive traffic. Approximately 8,000 people per year are killed in crossing streets in the United States. If the average American crosses five streets per day (3.5×10^{11} crossings per year), the risk per crossing is $8,000/3.5 \times 10^{11}$ or 2.3×10^{-8} . The risk from a diet drink is $1/6 \times 10^{-7}$, or 1.6×10^{-8} . Thus an average street crossing is about as dangerous as ingesting a diet drink.

Riding in automobiles involves a death risk of 2×10^{-8} per mile; thus a diet drink is as dangerous as one mile of automobile travel. Using a small car rather than a large car approximately doubles the risk. So purchasing a small car rather than a standard size car is at least ten times as dangerous (assuming 30 miles per day average driving) as ingesting one diet drink per day during the period of ownership.

Perhaps the best understood cancer risk is that of women not having an annual test for cervical cancer. The mortality risk from this neglect is about 6×10^{-7} per day, about 30 times higher than the risk of ingesting one diet drink per day.

The risk of one diet drink per day throughout life is readily calculated, using the Canadian study results, as $(1200/2 \times 10^8)$ risk per yr $\times 70$ yrs = 4×10^{-4} . Statistics show that the cancer risk varies considerably from one area of the country to another. For example, the risk is about 0.19 in New England compared to 0.15 in the south-east which has a similar age distribution. Thus moving from the south-east to New England involves 100 times greater cancer risk than ingesting one diet drink per day throughout life.

Approximately 7,000 Americans die each year due to fires, and it is estimated that about half of these could be saved if people would install smoke alarms in their homes (cost—about \$5 per year). Failure to install a smoke alarm is thus as dangerous as ingesting three diet drinks per day.

An endless list of comparisons could be put forward, but we have offered here a few examples that may be useful in helping understand the risk of saccharin. \square

Alternatives to sugar

The search for an ideal non-nutritive sweetener is almost a century old.

K. J. Parker outlines its history

SWEETNESS has long been known as a property possessed by many substances other than sugar, although their use as sweeteners has largely been confined to pharmacy, or precluded by toxicity. For example, chloroform, which was discovered in 1832, is still used for sweetening lozenges and linctus. The intensely sweet taste of liquorice root was known to the ancient Egyptians, an extract having been found in the tomb of King Tut (1500 B.C.). Although its strong liquorice flavour and pharmacological activity today limit its wider application as a sweetener, it is used in pharmacy, in some confectionery, and as a flavour potentiator.

The commercial development of artificial sweeteners dates from the discovery of saccharin in 1879, by C. Fahlberg and I. Remsen, followed by its manufacture five years later. In that year, the intense sweetness of *para*-ethoxyphenylurea was discovered by J. Berlinerblau, and within ten years, this compound was being marketed under the name of dulcin.

By the end of the last century sugar had become accepted as a basic food item, and its use and ready availability were taken for granted. Saccharin provided an alternative sweetener to sucrose, for use in times of sugar shortage, as a cheap substitute for carbohydrate sweeteners, or for inclusion in sugar-free diets. Its slightly bitter flavour and metallic after-taste, however, contrasting with the pure sweet taste of sucrose, reduces its acceptability. Consequently, the production of saccharin, which in Germany had reached 100,000 lb/year by the turn of the century and increased sharply during the two world wars, remained fairly static in Britain and Europe for the first half of this century.

Before 1940, inadequate nutrition had been a major concern of world health authorities. A decade later it began to be more widely appreciated that the converse—excessive or unbalanced food intake—could be equally injurious to health, possibly contributing to heart diseases, obesity and metabolic disorders. Since the need for essential dietary factors—vitamins, minerals, amino acids—was recognised, the emphasis for control of nutritional intake inevitably focused on carbohydrates and especially on sugar. Although the polemics of this particular view are still being debated, the substitution of sugar in the diet by a non-nutritive alternative has already

proved to be a painless and popular means of reducing carbohydrate—and hence, calorie—intake.

The demand for saccharin and its particular shortcomings meant that the market was ready for a more acceptable alternative. This was provided by a new sweetener, *N*-cyclohexylsulphamic acid, or cyclamate, discovered in 1937 by L. F. Audrieth and M. Sveda. In spite of its having only less than one tenth the sweetness of saccharin, the flavour of cyclamate was considered to be singularly pleasant and free from after-taste. Admixture of 10% saccharin effectively doubled its sweetness without introducing undesirable after-taste.

Legislation on sweeteners

The use of saccharin-cyclamate sweeteners, particularly in soft drinks, which currently accounts for some 70% of the synthetic sweeteners consumed, increased tenfold between their introduction and 1968. By then, concern was mounting over the potential hazard of unrestricted consumption of artificial sweeteners, especially by children, as a consequence of the widespread use of saccharin-cyclamate in non-dietetic foods and beverages.

A major manufacturer, Abbott Laboratories, had spent an estimated \$1 million on establishing the safety of this sweetener. Ironically, their disclosure that massive doses of cyclamate fed to rats was associated with the development of malignant bladder tumours in some of the test animals led in October 1969 to an immediate ban on the use of cyclamates in the United States. Great Britain, Canada and several other countries followed with equivalent legislation, though the restriction was not universal. Saccharin the only remaining permitted artificial sweetener, was subsequently removed from the Food and Drug Administration (FDA) list of food additives generally recognised as safe (GRAS list). Finally, in March of this year, following the results of at least six separate studies on rats in which cancerous tumours were positively identified, the use of saccharin in diet foods, cosmetics and non-prescription drugs was ordered to be withdrawn in the United States within two years, under the controversial 1958 Delaney amendment to the 1938 Food, Drug and Cosmetic Act. The Delaney clause makes mandatory the banning of the food use of any substance shown to induce cancer in any animal under any conditions.

It was against this background of an increasingly urgent demand for a safe non-nutritive sweetener that research into the chemical basis of sweetness was boosted in the late 1960s. In spite of the extensive research into the physiology and psychology of taste perception, however, it is still not possible to predict the chemical structure of an ideal non-nutritive sweetener, or even that a substance will be sweet. Many types of compound are known to taste sweet, but in every class the initial discovery of sweetness was accidental. There seems to be no common molecular property which can be used to predict the ability to elicit a sweet taste.

Theoretical models have been advanced for the molecular basis of sweetness, the most comprehensive being that of Robert Shallenberger of Cornell University. In its most recent form, his model accounts for the perception of sweetness in terms of a three-point interaction with the taste receptor protein, involving a proton donor, a proton acceptor and a lipophilic group sited at the points of a precisely specified triangle. In spite of the ingenious explanation of the sweetness of known compounds, however, not all compounds fitting the model are sweet and the theory cannot be used to predict or construct radically new sweeteners.

Nature and nurture

Research into new sweeteners thus takes the form of molecular variations on a known structural theme. Alternatively, intensely sweet substances of natural origin can be sought and characterised. Either approach, however, is ultimately subject to the requirement that the candidate sweetener should meet the increasingly rigorous toxicological safety standards imposed by the public health authorities, before it can be permitted for use as a food additive. Since toxicity cannot be predicted on the basis of molecular structure either, the search is inevitably speculative.

Since the ban imposed on cyclamate, Abbott Laboratories have been continuing to sponsor research into the toxicology and safety of cyclamate, and in November, 1973, petitioned the FDA for its reinstatement on the grounds of need and in the light of further experimental evidence. It is argued that the single piece of evidence indicting cyclamate was not substantiated by subsequent studies. With the

eventual withdrawal of saccharin there will be no permitted sweetener on the US market, a situation in which the FDA may be obliged to reconsider rescinding the earlier ban on cyclamate, though so far the Abbott petition has not been allowed.

New synthetic sweeteners

Several candidate sweeteners are being developed, but very little published information is available. The market is highly competitive, and no wonder for production of a multi-billion dollar sweetener is the prize.

In addition to cyclamate, two other non-caloric sweeteners, Aspartame and β -neohesperidin dihydrochalcone, have been under consideration by the FDA, though neither has yet been declared wholly acceptable. The intense sweetness of the methyl ester of the dipeptide α -L-aspartyl-L-phenylalanine was discovered by James Schlatter working in the G. D. Searle & Co. laboratories and reported by R. H. Mazur, J. M. Schlatter and A. H. Goldkamp in 1969, though the compound had been first synthesised three years previously within ICI. Searle filed a patent for the use of this compound as a sweetener, while ICI patented the cyclohexyl analogue at almost the same time.

Since this discovery, several hundred closely related compounds have been synthesised, many of which have proved sweet. However, Searle decided to develop and market the original dipeptide methyl ester under the name of Aspartame, in conjunction with the Japanese Ajinomoto Company, which had independently developed a cheaper synthetic route. The possible formation of the cyclic self-condensation product diketopiperazine, formed in soft drinks on storage, for example, resulted in Searle voluntarily withholding the product from the market, even though qualified approval had been granted by the FDA in 1974 for the use of Aspartame as a table sweetener. Then in December, 1975, the FDA rescinded its earlier order indefinitely, pending further review of the evidence on safety, in spite of the removal of earlier fears on the carcinogenic properties of diketopiperazine.

In a study of the bitter principles of citrus fruit rind, R. M. Horowitz and B. Gentili discovered that the bitter flavanone naringin from grapefruit peel could easily be converted into the corresponding dihydrochalcone which by contrast was found to be intensely sweet, a property shared by a number of related dihydrochalcones. Of these β -neohesperidin dihydrochalcone was selected for toxicological and metabolic studies by the Southern Regional Laboratory of the US Department of Agriculture. The pilot plant production was also developed making possible its

manufacture in ton quantities when required. Although its low solubility in water and the delay in taste perception make this sweetener unsuitable for many applications, once granted food additive status it could be used for sweetening chewing gum, toothpastes, pharmaceutical preparations, etc where such properties are not a disadvantage.

A new class of sweeteners, the oxathiazinone dioxides, was reported in 1973 by K. Clauss and H. Jensen of Hoechst. These, like saccharin and cyclamate, contain the sulphonamide group and a lipophilic part. The β -methyl derivative, which has been reported to have the best flavour profile, is being developed by Hoechst under the name Acetosulpham. It has ap-



Wild serendipity berries

proximately one third the sweetness intensity of saccharin.

Although several amino acids are known to taste sweet, it was discovered accidentally, by Kornfeld of Eli Lilly and Co., that some 6-substituted D-tryptophan derivatives are intensely sweet. Of these, 6-chlorotryptophan, reported to be approximately 1,000 times as sweet as sucrose, has been selected for development as a sweetener by the company.

A systematic study of the relationship between taste and chemical structure of a series of terpene oximes and related compounds has been undertaken by a research team at Stanford University led by E. M. Acton. The intense sweetness of the α -syn-oxime of perillartine was first reported in 1920 by Furukawa in Japan, although this compound is unsuitable for use as a sweetener owing to its low water solubility. However, a closely related oxime has been proposed as a suitable candidate sweetener by the Stanford group.

Since the early discovery of the intense sweetness of alkoxyaromatic amines—examples of which are Dulcin and, more recently, the deep yellow P 4000, used briefly as a sweetener in

Holland during the war—numerous intensely sweet arylamines have been described. These compounds are generally toxic, and are thus unlikely to be selected for further development. However, A. Zaffaroni, of the Dynapol Corporation of America, has conceived the idea of linking a sweet molecule to a non-absorbable, inert polymer in such a way that the taste interaction of the sweet part is unaffected. Not being metabolised, the sweetener cannot manifest systemic toxicity and is non-caloric.

Why sugars and polyhydric alcohols reach a limiting level of sweetness with sucrose, while non-hydroxylic compounds of similar molecular weight can be several orders of magnitude sweeter



Thaumatococcus plants

can be explained by the absence of a lipophilic group from the sugar molecule. Pursuing this theme, Michael Lindley and Gordon Birch of the National College of Food Technology, Reading University, prepared several methyl ethers of sucrose but none was found to be particularly sweet. This approach was vindicated, however, with the discovery by Leslie Hough and S. P. Phadnis of Queen Elizabeth College, London, of the intense sweetness of tetrachlorogalactosucrose, while working in collaboration with Riaz Khan of Tate & Lyle, on deoxy- and halodeoxysucrose derivatives. They subsequently showed that sweetness reaches a maximum in 1',4,6'-trichlorotrideoxygalactosucrose, found to be approximately six hundred times sweeter than sucrose. It has a pure sweet flavour closely similar to that of sucrose. Although it has been shown not to be toxic to rats and to be non-caloric, its suitability as a non-nutritive sweetener has yet to be established.

Natural sweeteners

The occurrence of high-intensity sweeteners in nature has long been recognised, many having been used traditionally for generations: for ex-

ample, glycyrrhizin from the roots of the liquorice plant, the fruit of the South Chinese Lo Han Kuo and the leaves of the Paraguayan shrub *Stevia rebaudiana*. Stevioside, a triterpene glycoside, which is readily extracted from the leaves of *S. rebaudiana*, is being produced on a commercial scale in Japan by the Toyo Menka Karishma Company. Stevioside, which is approximately 300 times sweeter than sucrose, is not a permitted food additive elsewhere.

More recently the serendipity berry (*Dioscoreophyllum cumminsii*) was identified in a broad survey of sweet fruits undertaken by George Inglett and J. F. May, Department of Agriculture, in 1968. Its berries are intensely sweet owing to the presence of the protein monellin which is approximately 2,000 times as sweet as sucrose. A similar protein, thaumatin, has been isolated from the fruit of a West African plant *Thaumatococcus daniellii* by H. van der Wel and K. Loeve of Unilever. This has proved to be the sweetest substance known, being

around 4,000 times sweeter than sucrose. The extraction, purification and properties of both monellin and thaumatin have been studied extensively, in particular by Tate & Lyle, who are able to produce thaumatin in kilogramme quantities. However, the toxicology of thaumatin has not been fully evaluated, though, being a vegetable protein which has been consumed for generations, it seems unlikely that it would have harmful side-effects.

Similar considerations would seem to apply miraculin, the glycoprotein extracted from the berries of the bush *Synsepalum dulcificum*, also native to West Africa. An extract of this fruit, which has the property of tasting intensely sweet only in the presence of acids, was marketed for a time by the Miralin Corporation of America who established extensive plantations in several countries to produce the extract. However, FDA approval for this product was denied in 1974 pending toxicology testing, with the consequence that it is not now commercially available.

Naturally occurring sugars and sugar alcohols, such as fructose, xylitol and maltitol, are also being evaluated as alternative sweeteners, though since they are metabolised, they cannot be regarded as non-caloric. Xylitol, produced from birch wood, is available at ten times the price of sugar. It is finding use as a sweetener in chewing gum, though it is not a permitted sweetener in Britain and, following recent evidence of potential carcinogenic activity, may be prohibited in the US.

In spite of the intense interest worldwide in developing alternative sweeteners, the ideal non-nutritive sweetener—water soluble, chemically and thermally stable, of pure flavour, non-toxic, and of high sweetness intensity—has not yet been discovered and may still be some years off. In the meantime a less satisfactory compromise seems inevitable. □

K. J. Parker is General Manager, Research, with Tate & Lyle Limited.

No soft options in Carter's energy research policy

ENERGY policy was a major plank in Jimmy Carter's campaign platform during the 1976 Presidential election. In a year when the rising cost of imported oil was causing increasing economic concern, and the late E. F. Schumacher was telling capacity audiences that "Small is beautiful", Carter promised that, if elected, he would shift the emphasis of US energy technology away from a prime dependence on nuclear fuels and towards non-nuclear alternatives, in particular renewable resources such as solar power.

But like many other campaign promises—for example that to reduce the budget of the Defense Department, now scheduled for a 9.4 per cent increase to \$117.8 billion in fiscal year 1979—Carter's energy plans have, in the face of established institutional positions and interests, undergone considerable revision in his first year of office.

The President has maintained his firm stand on the dangers of proliferation resulting from the production of plutonium by fast breeder reactors, in particular demanding the closure of the Clinch River liquid metal fast breeder reactor at Oak Ridge, Tennessee.

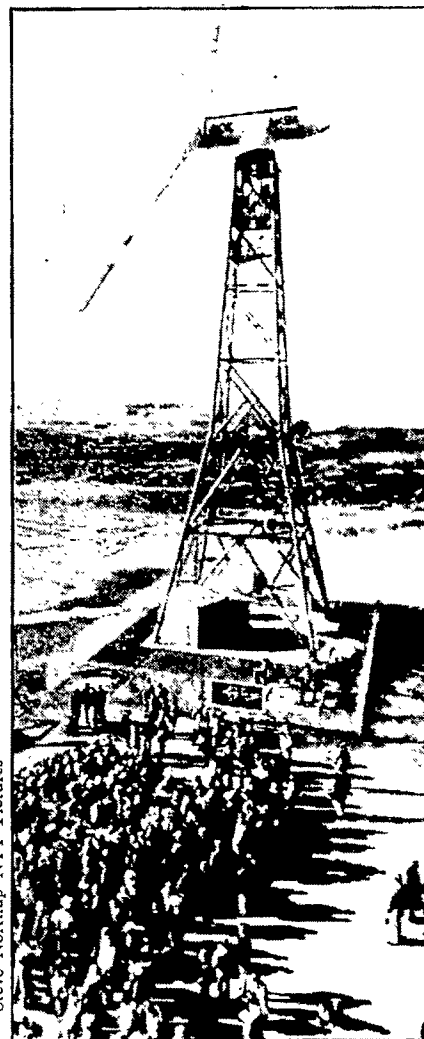
But the administration is still pursuing a vigorous nuclear policy through other options, including possibly a major design project for a thorium-

cycle reactor. And already last year, following, in particular, moves to speed up the licensing of new reactors, Carter's earlier claims that he would turn to nuclear energy only as "a last resort" were beginning to wear thin.

Further confirmation that energy policy has changed little comes from analysis of the Department of Energy's proposed research and development budget for the fiscal year 1979. In presenting this to Congress two weeks ago, President Carter has suggested that the overall level of energy R&D remain roughly static at \$2.7 billion.

The budget contains considerable increases in commitments to research on synthetic fuels (another area in which campaign promises seem to have been forgotten). In contrast, resources for research into renewable energy sources show little change. It is proposed, for example, to raise the budget for windpower research from \$37 million to \$41 million; and solar power research, which has grown from \$4 million in 1974 to \$193 million in 1977 and \$303 million last year, will increase only to \$309 million. The increases in these two topics scarcely keep up with the expected increase in the cost of living.

There are, admittedly, some important shifts in philosophy reflected in the budget proposals. Perhaps the



The inauguration of the US National Aeronautics and Space Administration's windmill in New Mexico

Steve Northrup NYT Pictures

most significant is a hefty 27% increase in funds for conservation R&D, under which funds for research on topics such as motor efficiency and energy storage will be increased from \$301 to \$381 million.

In addition, the proposed closure of the Clinch River fast breeder project, the subject of a furious political battle last autumn, after which Carter vetoed the authorisation bill and is yet to decide what to do with a supplemental appropriation of \$80 million passed by Congress, would save \$150 million, creating an apparent swing in emphasis from nuclear to non-nuclear technology.

But apart from the Clinch River project, there has been little change in relative priorities. Indeed if the Clinch River funds are extracted from the 1978 budget figures, the proportion of R&D expenditure going to other nuclear projects actually increases between 1978 and 1979, from 45.8 to 46.4 per cent of the total for research and technology development.

There is therefore still a strong continuation of pre-Carter policy directions. Dr John Deutch, who recently moved from chairman of the department of chemistry at the Massachusetts Institute of Technology to become director of the Office of Energy Research, told a House Subcommittee last week that "the strategic approach to our nation's energy R&D efforts has been evolving over the last several years".

But if there has been no obvious shift in priorities, there has been a significant change in strategy. In presenting the President's proposed R&D budget two weeks ago, Dr Frank Press, director of the Office of Science and Technology Policy, said that the administration was trying to shift the emphasis of R&D funding away from development projects—considered the responsibility of private industry—towards more basic research.

Dr Deutch shares this strategy, although he puts it slightly differently. In an interview with *Nature* last week he pinpointed the problem in energy research as lying not at the level of basic science, in which the department's position is relatively strong, but at an intermediate point in the R&D spectrum which he identifies as "advanced development work". In this he includes fields such as geology, and chemical and process engineering.

Dr Deutch also stresses the need to maintain flexibility in research programmes, developing a "hedged" strategy and keeping options open, so that the department can respond quickly both to new research potentials, and to changing parameters affecting energy supply.

In the nuclear field, for example,

one criticism of the Clinch River project has been that its massive funding has required putting too many eggs into one basket, and that the project was an attempt to move too fast in a single direction when revised predictions of future energy demand are indicating that the speed may be premature.

In contrast, the administration is suggesting that a close look should be taken at a wide range of options in the nuclear field, and at an earlier stage than project development. Thus within an overall R&D budget that is virtually static, funding for "basic energy sciences" is being increased by 20 per cent from \$177 million to \$212 million, and for nuclear research and applications by 22 per cent from \$227 to \$278 million. 'Advanced technology and assessment projects', which will involve a mechanism for monitoring and providing a critique of R&D programmes, will be increased almost three-fold, from \$8 million to \$21 million.

One alternative that President Carter is known to be particularly enthusiastic about is the thorium cycle reactor which, by using the thorium-uranium conversion process rather than the uranium-plutonium process, would be much safer than the conventional fast breeder.

Carter's enthusiasm for the thorium cycle is not shared by the environmentalist lobby, many of whom feel that it still fails to meet adequate safety criteria, at least in comparison with once-through light water reactors. But the Department of Energy is expected to announce soon a major \$15 million project for the conceptual design of a 650 to 900 megawatt reactor, to fill the programme gap left by terminating the Clinch River project; and the thorium cycle is a high contender for this new design.

Dr Deutch said last week that he felt

there was still not sufficient stress on solar research and development. But in line with the administration's general approach to R&D, funds for "production, demonstration and distribution" of solar energy supply are being reduced from \$87 million to \$64 million in 1979.

The decision to limit the growth of solar research funds has not been a popular one in Congress, where solar energy, with its apparent promise as a pollution-free, virtually infinite resource, carries a strong and populist appeal. Pointing out in the House of Representatives that the cutbacks in the solar energy programme were more than the salary increases requested by the Department of Energy, Representative Louis Frey Jr of Florida claimed that the budget could be characterised as "one giant step backward in energy research and development."

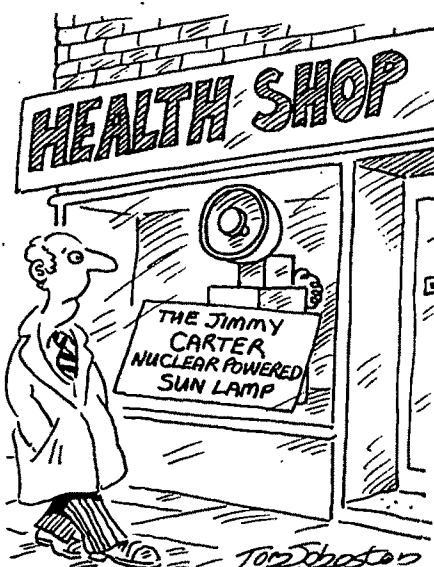
There will be a powerful campaign to increase solar energy's share of the R&D budget when the latter is debated in committee. And the campaign will receive added fuel from a shortly-expected report from Congress' General Accounting Office, which is thought to suggest that as much as \$600 million—almost twice the amount proposed by the administration—could be fruitfully absorbed into existing and proposed solar energy research projects.

In itself, the apparent conflict between the administration and Congress is not too important. In areas known to have enthusiastic support on Capitol Hill, the administration is often claimed to feel safe in going for a low budget knowing that it will be increased, and that it can put in higher bids in areas which Congress traditionally cuts back.

But in this case the stakes are higher than usual. President Carter has publicly asked to be judged by the success of his energy policy; hence his frustration at the stalemate on energy legislation, and the important symbolic role that it has taken on. However, in cutting back on solar energy research and continuing to emphasise nuclear options, whatever the administrative and scientific logic, he stands to lose the confidence of precisely those whose support he needs to push through his whole energy policy.

As one environmentalist who has been closely involved with recent debates said last week, in words that have been echoed across a range of issues: "I'm obviously disappointed that Carter's going back on his campaign promises, but I'm more disappointed that he won't admit what he's doing, that he won't come out and say that he has changed his mind."

David Dickson



Bangladesh sets up institute of nuclear agriculture

THE Vice-President of the Bangladesh Republic officially inaugurated the Institute of Nuclear Agriculture (INA) last month. This Institute, set up in collaboration with the International Atomic Energy Agency (IAEA), is the fourth large one of this kind to be built. The others are in Yugoslavia, India and Brazil. The Institute has been established in the premises of Bangladesh Agricultural University with the objective of identifying and solving the basic agricultural problems of the country through interdisciplinary approaches employing both nuclear and conventional techniques.

Agriculture is the mainstay of the Bangladesh economy. Farm products alone account for about 55% of the Gross Domestic Product (GDP), so the importance of boosting productivity can hardly be over-emphasised at a time when the country is facing the joint problems of food shortage and a fast growing population.

The initial research programmes of the Institute are in plant physiology, plant genetics, soil science, and ento-

mology, with emphasis on improving varieties of rice, jute, wheat, pulses, tomato and oil seeds. INA has already achieved some encouraging results in evolving new rice varieties, such as IRRATOM-24 and IRRATOM-38 through irradiation technique. They are capable of higher yield and early maturing than mother variety IRRI-8. Similarly, the two new strains of jute known as ATOMPAT-8 and ATOMPAT-38 have been found superior to the mother, D-154.

Mr Hellio F. S. Bittencourt, Deputy Director General of IAEA, addressing the inaugural ceremony said that the application of nuclear techniques in agricultural research was by no means a luxury for the developing countries; it was the base proven method for solving certain practical problems of agriculture.

The Swedish International Development Agency (SIDA) has provided a grant of over one million US dollars towards the cost of equipment, advisory service and fellowships, IAEA being the executant agency.

M. Kabir

Less paper, more money for US scientists

DR FRANK PRESS, director of the US Office of Science and Technology Policy, has launched a major campaign to encourage Government agencies to reduce the amount of time that university scientists receiving federal support are required to spend on paperwork.

Speaking in Washington last week, Dr Press said that the money that could be saved in this way might amount to millions of dollars for every university.

"One of the goals of this administration is to make universities perform better the basic research which the government subsidises. One way of reducing paperwork and overheads, for example, would be to award longer-term grants, so that scientists do not have to be writing new research proposals every three months or so."

Dr Press said that the Department of Health, Education and Welfare, which is responsible for the research carried out by the National Institutes of Health, is already looking at ways in which it can help universities by reducing paperwork and streamlining regulations about grants.

"I have also written to NASA (the National Aeronautic and Space Administration) and to NSF (the National Science Foundation) asking them what their plans are on this," he said.

Dr Press said that, under the current legislative framework, the administration could not guarantee support for a particular research project over a two or three year period, since the research budget had to be approved annually by Congress.

"However we can say to someone that we intend to support his work for a given number of years, and that if he or she sends in a report on the progress of his work at the end of each year which is satisfactory, we will agree to further funding." The Science and Technology committee of the House of Representatives is already looking into the possibility of multi-year authorisations as a way of stabilising the research-funding process, he said.

Dr Press also said that although there was little support in the administration for setting up a Department of Science, there was growing interest in establishing a Department of Technology and Industrial Development.

"Many countries, such as Germany, England, France and Australia, already have such ministries. This could be an interesting direction for the administration to go, particularly since there is an increasing sensitivity at Cabinet level to the problems of industrial innovation."

David Dickson

The facts about Kosmos-954

RECOVERY of the fragments of Kosmos-954 is proving, not surprisingly, a lengthy operation, and although some sizeable pieces have apparently been located and taken for examination to White Shell, Manitoba, it may be some considerable time before any hard facts are available about the type of satellite and reactor actually involved. Although the figure of 50 kg U-235 has been tossed around fairly freely, this is simply an assumption the reactor involved was of *Romashka* type. This is the only Soviet reactor of comparable type ever described in open publications; accordingly, it is the only basis for speculation. The *Romashka* has a 40 kW thermal yield and was, indeed, designed for space use, and assuming that such reactors are still used aboard certain Soviet satellites, it is possible to work out tentative figures for radiation hazards for this, and possible future, satellite crashes.

Three scenarios have been discussed among the experts. The first—break-up of the satellite in the stratosphere—requires the use of additional data. This comes from the US Navy SNAP-9A, powered by a plutonium source, which disintegrated before entry into orbit in 1964. On this basis, assuming an initial charge of 50 kg U-235—and 100 days in orbit before disaster, one arrives at the comforting figure that six months after the incident the residual radioactivity would be of the order of 10^{-9} of the International Commission for Radiological Protection safety level. (This is the concentration of airborne radioactivity that could be breathed 24 hours a day without harm.)

For break-up on impact, the downwind distance to which the UK Medical Research Council emergency reference levels extend works out at the order of a few km. (This means in effect that beyond this distance the hazards of mass evacuation are considered to be greater than the risk from radiation). For impact in one piece, if no chain reaction occurred, initial radiation levels would be expected of 1 rad/h at 200 m from the site, 100 millirad at 500 m, 10 millirad at 1500 m. Had the impact occurred in a populous area, evacuation would have been necessary up to 2 km, if no intervening shielding (e.g. masonry) were present. Chain reaction is unlikely, since the mass of uranium used in such satellites is normally subcritical, and special methods, e.g. a reflector, must be used to keep sufficient neutrons within it to maintain a chain reaction. Such a reflector would virtually certainly become

detached and destroyed during uncontrolled descent.

However comforting or worrying these figures, there is no doubt that the gloomy prediction of Ecclesiastes that "they shall be afraid of that which is high" has taken on a new and urgent meaning. President Carter has already expressed his willingness to be a party to a treaty banning the use of reactors from orbit. For the Soviet side, Academician Leonid Sedov has said in a TASS interview on Soviet radio that the errant satellite was in no way the "flying nuclear bomb" which some "absurd rumours" would make it. There were not and could not be any weapons aboard, he said, and those who spread such tales were trying to undermine the basic principles of international cooperation in the exploration and peaceful use of outer space.

In spite of Sedov's reassurances, it seems likely that Kosmos-954 did have certain military potentialities. Technically, it was a low orbit satellite for ocean surveillance. The presence of a reactor aboard (as opposed to a weaker isotope source) implies the use of powerful radar for monitoring ship movements, which would suggest that the terms of reference of the international agreements on the peaceful uses of both space and atomic energy were being somewhat stretched. Any future agreement on reactors in orbit might well have to re-examine the definition of 'peaceful' in this context.

Doubtless as a further reassurance, Sedov presented the official Soviet view of the accident: a sudden depressurisation of the satellite while beyond the range of Soviet tracking facilities. Since depressurisation was so rapid, it is assumed that Kosmos-954 collided with "some other object of natural or artificial origin".

Strangely, Sedov gave the significant date as January 6—the date of the "depressurisation". Yet according to US observers, the satellite was already misbehaving on December 17. The two reports are not incompatible—if the satellite was already out of control, it might well have come into contact with some other artificial object or debris which it normally should not have encountered. This, however, is not Sedov's story: he maintains that the on-board systems became inoperative only as a result of the January 6 impact—one more minor mystery to add to the story of Kosmos-954.

One fact, however, has already emerged quite clearly—the cost of the search and recovery operations. According to Canadian Prime Minister Pierre Trudeau, by last weekend this had reached one million dollars and was rising daily.

Vera Rich

Britain's big science on the baseline

AFTER four months in office, Professor Geoffrey Allen, Chairman of the UK's Science Research Council (SRC), is pleasantly surprised that the Advisory Board for the Research Councils (ABRC) is not as hostile to basic science as he had been led to believe by his predecessor, Sir Sam Edwards.

Professor Allen, speaking at a British science writers' luncheon, said that in spite of the proposed 1.7% annual cut in the SRC's budget over the next four years, announced with the science vote last month, the outlook for science in the UK is looking slightly up. His cautious optimism is founded on the ABRC's decision to award the SRC greater shares than it could expect, in proportion to its annual expenditure, of the extra £4 million added to the science vote last October, and of the £4.5 million to be spent on capital work in 1978/79.

A beneficiary of that optimism could be 'big science', long since neglected for research designated of national importance. "Nuclear physics, astronomy and space research have been battered" admits Professor Allen. For nuclear physics, in particular, he agrees that funding is now at the minimum viable level—almost. "We've nearly reached the baseline" he says, "which we must maintain to support a UK presence in nuclear physics".

And Professor Allen seems determined to have a say in the fate of British nuclear physics. Last December, he was at his own request appointed to the Council of the European Centre for Nuclear Research (CERN) in Geneva, now the major recipient of Britain's funds for high energy physics. He has already expressed his view that CERN's annual budget should be planned to remain at a plateau of about Sw Fr 560 million. The budget for 1978 is Sw Fr 615 million and many countries would like to see future budgets stabilise at about Sw Fr 600 million.

Not all British nuclear physicists, however, are in agreement with Professor Allen's baseline. Dr G. Stafford, Director of the UK Rutherford Laboratory thinks that a Sw Fr 560 million budget at CERN "will mean losing out on basic experiments". Such a low sum, he claims, "takes no regard for future development. By the 1980s existing machines will be old and at that level of funding we would not be able to replace them". On one point, though, Allen and Stafford do agree; that if future projections for the total UK nuclear physics budget are met, involving a 25% cut in spending over

the next five years, then there would be insufficient funds for a home-based programme and UK nuclear physics would be at an end. Stafford feels that the funding is at an absolute minimum.

The fate of high energy physics, however, is being pursued on another level. At the end of this week, Shirley Williams, Secretary of State for Education and Science, and a powerful lobbyist for the interests of scientists in the UK cabinet, will visit CERN.

The state in Britain of the other big science—astronomy and space science—is perhaps not quite so critical as that of high energy physics. Provided that there is no large cutback in funds over the next few years, radio astronomers should be able to maintain a competitive programme. Plans for a millimetre-wave radio telescope already have SRC approval: all that is now needed is Treasury approval for the £4.5 million it is likely to cost. And Jodrell Bank has plans to go ahead with extending its multi-telescope interferometer at an estimated cost of £2 million.

In astronomy it is the X-ray astronomers and space scientists who are showing most concern over their futures. Professor Ken Pounds of Leicester University claims that British X-ray astronomy is in a "fairly critical state". Its international reputation is higher than it has ever been but the prospects for the future are poor unless funding can be maintained at least at the present level. "If the UK is to get the best out of its subscription to the European Space Agency (ESA), then it must have a comparable home programme". But ESA can only fund one major new project in any particular discipline every two years, so each discipline is catered for approximately once every ten years. At the moment, for example, ESA has only one satellite for X-ray studies, EXOSAT. Therefore the UK must be able to bid for space on other satellites including NASA's Space Shuttle. This means maintaining a home based programme to the tune of £5-6 million.

Professor Allen's concern, however, is not entirely with the problems of 'big science'. He sees as one of his immediate priorities "making Sam's plans succeed" by continuing to give special attention to areas of national importance including microelectronics, marine technology and polymer engineering and making sure that schemes to improve collaboration between industry and universities are implemented.

Judy Redfearn

Skylab coming down

OFFICIALS at the US National Aeronautics and Space Administration are concerned that Skylab, last used in 1974 and still, at 85 tons, the largest manmade object in earth orbit, could fall to earth later this year.

Originally it was planned that Skylab would stay in orbit until 1980, when a manned space shuttle could attach a rocket motor to it that would send it into a higher orbit, or initiate a controlled re-entry.

Recent calculations, however, indicate that Skylab, which cost \$294 million to launch, could crash into the atmosphere later this year, and NASA officials are now working on a plan to command the still-operable steering rockets to send Skylab into a controlled tumble that would speed up re-entry.

The manoeuvre would be carefully calculated to bring the station back to earth on a steep descent over a broad area such as the Indian Ocean or the South Pacific. □

Letter from Sakharov and Meiman

IN October 1977, the US Association for Computing Machinery informed their Soviet opposite numbers that they would not cooperate with or sponsor international conferences in the Soviet Union until the Soviet attitude to human rights, exemplified by the case of Anatolii Shcharanskii, improved. Recently the ACM received the following message:

To the Association for Computing Machinery; to US scientists and engineers in computer technology

Distinguished Gentlemen, We'd like to express to you our deep gratitude for your resolute actions on behalf of Anatolii Shcharanskii. You have hit just the right nail. The Soviet authorities extremely appreciate the cooperation in science and technology, thus, there is nothing to induce them so factually and effectively as a refusal to maintain

this cooperation.

We'd feel happy if your actions were followed suit by physicists on behalf of Professor Yurii Orlov and Andrei Tverdokhlebov, by biologists and medical specialists on behalf of Sergei Kovalev, as well as by writers and editors on behalf of Aleksandr Ginzburg.

Your courageous and noble stand is not simply ethically the best but the only practical one. Do not believe and do not take seriously any assertion that your decision allegedly could only embitter the Soviet authorities and aggravate the situation of Soviet scientists. Do not doubt that your human and professional solidarity will bring positive results.

*Nobel prize winner, Academician
A. Sakharov*

*Professor of Mathematics
N. Meiman*

CORN (maize) is the principal grain crop of the United States. Cash receipts from corn in the US in 1969 were nearly twice as much as for soybeans, and almost three times as much as for wheat. Peanuts (groundnuts) netted only 7% of the dollar value for corn. They have since become nationally prominent for presidential reasons. Peanuts, like soybeans, are legumes; they fix nitrogen and their seeds are good sources of proteins. Peanut protein is better balanced with respect to amino acids than is corn protein, but it is low in lysine, methionine and threonine.

Corn, in damp weather, and peanuts are good hosts for the growth of a mould, *Aspergillus flavus*, that produces aflatoxin, a potent carcinogen. The Food and Drug Administration (FDA) and the 'Delaney Clause' smiled benignly on aflatoxin, which is produced by God rather than the chemical industry, and is therefore not bannable. An 'action level' of 20 ppb of aflatoxin is used as a guideline for regulation. This level is carcinogenic in laboratory animals. In 1974, the FDA proposed to replace this level by a tolerance of 15 ppb for peanut products. Presumably this was intended to lower the cancer risk from aflatoxin somewhat, but the modest retreat is scarcely in accordance with the principle of total exclusion so sternly embodied in the Delaney Clause.

On 14 November, an FDA spokesman announced that 66 life-time cancers per 100,000 persons could develop from normal or expected ingestion of aflatoxin-contaminated pea-

nut and corn products. Presumably this figure would correspond to about one case per 100,000 persons per year. It was based on a combined estimated consumption of 2 ppb of aflatoxin from peanuts and 10 ppb from corn

Corn and Peanuts



THOMAS H. JUKES

products. This cancer rate may be compared with about 33 per 100,000 for lung cancer deaths, usually largely attributed to cigarette smoking, and about 168 for all US deaths from cancer. The estimate for aflatoxin corresponds to about 2,000 cancer cases for the entire US population.

I assessed the cancer hazard from diethylstilbestrol (DES) in beef production as not more than one case in 2,500 years in the entire US popula-

tion, based on pessimistic figures. This estimate, for some reason or other, was not challenged by the FDA lawyers who cross-examined me on 4 November at the hearings on DES in meat production. Perhaps this rather striking difference between hazards from DES and aflatoxin should be pondered by the FDA in setting priorities on bureaucratic regulations.

Aflatoxin should also be considered by vegetarians who tell us that we should eat corn, rather than feeding it to cattle, because we need to be less wasteful of food. The same school of thought advocates eating plant proteins, such as those of soybeans and peanuts, as replacements for beef and pork. However, when cattle and pigs eat corn and other feeds that contain injurious substances, they may filter them out, excrete them or metabolise them, especially if they are water-soluble. Being in a high position on the 'food chain' may have its advantages, even though we are frequently told that this is not the case.

The most active substances that inhibit mould growth belong to the antifungal group of the currently-criticised chemical pesticides. Also, oddly enough, the toxic effect of aflatoxin in rats can be reversed by dosing with DDT.

Two years ago an outbreak of hepatitis, with more than 100 deaths, was reported in India, associated with the consumption of corn that was contaminated with aflatoxin. Affected people could have consumed 2 to 6 mg daily for a month. About 1000 persons who recovered are being followed for liver cancer.

correspondence

GDR's state of science

SIR,—Under this presumptuous title your journal, which has built its reputation on a claim of fair and objective reporting, saw fit to publish the 'impressions' of an anonymous correspondent of his visits to unnamed biochemistry and microbiology laboratories in an undisclosed city of the GDR (13 October, page 548). The picture on the same page intimates that it might have been Leipzig. As a biochemist at the Karl-Marx University of Leipzig I feel that the readers of *Nature* deserve substantial factual information to do justice to the heading chosen by the editors.

The state of biochemistry is of course part of the general development of sciences in the GDR. In the last ten years alone funds for science have increased more than threefold and the percentage of the GNP going to science and technology has increased from 2 to 5. The life sciences have had their fair share in this development. As far as biochemistry is concerned it may suffice to mention that of the seven departments in medical faculties six are housed in new buildings (Rostock, Berlin, Leipzig, Halle, Jena, Magdeburg). In the Academy of Sciences there have been founded eight institutes of life sciences with a sizeable representation of biochemistry, in particular in the Institut für Molekularbiologie, Berlin-Buch, and the Institut für Biochemie de Pflanzen, Halle. All establishments are headed by prominent scientists rather than by administrators. The development of research in the life sciences is largely based on prognostic documents, worked out by some hundreds of scientists themselves, which have been accepted by government as a framework for implementation.

The vast majority of scientists in the GDR go along with the tenets held by most thoughtful scientists elsewhere—that freedom of research is tied indissolubly to social responsibility. All scientists loyal to the GDR have equal chances regardless of creed and there is certainly no pressure to join the Socialist Unity Party or any other. As far as the spirit of young biochemists is concerned all I have experienced is enthusiasm rather than despondency. There is a general feeling of social security and optimism shared by the scientists with all citizens of the GDR which is based on the record of uninterrupted progress of our socialist society which does not know economic

crises or unemployment. There is of course no denying that we wish to have more foreign exchange at our disposal for more equipment, literature and travels. But we know that this depends in the first line on the efforts of our workers to increase export.

As far as conditions at the Karl-Marx University are concerned, scintillation counters, analytical and preparative ultracentrifuges from internationally renowned manufacturers as well as the internationally leading biochemical journals are in fact available. The Karl-Marx University maintains an effective cooperation with British universities and biochemists.

For many years there has been a regular exchange of staff and students, biochemists included, between the Karl-Marx University and the universities of Leeds, Manchester and York. Two biochemists of the Karl-Marx University, one of them myself, are members of The Biochemical Society of Great Britain.

The growth of biochemistry is reflected in the number of members of the Biochemische Gesellschaft der DDR, whose membership has doubled over the past few years and now totals 610. The scientific life is an active one and a multitude of national and international meetings are held including the Federation of European Biochemical Societies (FEBS) Advanced Courses. It is to be hoped that a good many readers of *Nature* will participate at the 12th FEBS Meeting to be held in Dresden, 2–8 July, 1978 and will be able to judge for themselves both the general state of life and of science in the GDR.

E. HOFFMAN
Karl-Marx Universität, Leipzig, GDR

Discarding the diet-heart hypothesis

SIR,—No, Mr Rivers (3 November, page 2), I am not a heretic, I am a scientist. Science is for me a religion both because it opens an endless wonderland and because it works. It is, in a needy world, a way to solve problems, as are Buddhism and Christianity and Judaism and the other religions, each in their own way. The way science works is quite simple but not widely understood. Using the best available evidence the scientist formulates an hypothesis and then he and his peers set out to disprove that hypothesis. We never prove in science, we advance to new understanding by

disproofs. The hazard, as Platt has so eloquently said in his essay 'The step to man', is that scientists may become so enamoured with some favourite hypothesis that they never test it. Or they become intrigued with the testing hardware and dawdle or they refuse to accept the disproving results of good tests. The last has happened with the diet/heart hypothesis. A noisy clutch of scientists is defending, excusing, delaying, procrastinating over the decision to discard a disproven hypothesis. Their tactics prevent progress.

Sir Andrew Huxley was lately quoted (8 September, page 95) as saying that the most sinister behaviour for scientists is to refuse to test an hypothesis (say the inheritance of intellectual ability) because it would be thought socially or politically wicked to raise a question whose answer might disagree with some preconception. No less sinister is the tactic of the diet/heart enthusiasts who, by their noisy propaganda hope to coddle and prolong the diet/heart hypothesis. That hypothesis has been shown wrong by scientific method. I believe that because I believe in the scientific method.

The heretics, Mr Rivers, are selling corn oil.

GEORGE V. MANN

Nashville, Tennessee

Research and culture

SIR,—It is sad, when it comes to defending support for basic science, that *Nature* editorialists should resort to stating the obvious, that is that basic research is a cultural activity ('How much further can the pendulum swing?' 27 October, page 743).

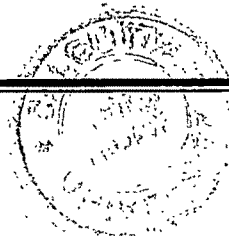
Sad but not surprising, considering how many educated persons, including scientists, seem to ignore this fact or are confused by the distinction between civilisation and progress. Civilisation is culture. Encouraging cultural activity is maybe the best investment for the future of a nation; it is an investment in enlightenment, a non-depreciating quality (whether or not research comes up with the cure for cancer—that would be progress).

After talking with friends in the British theatre, however, who have been out of work for a while now, it might not be such a good idea, after all to remind the government that research is a cultural activity.

GIUSEPPE ZACCAI
NORINE ZACCAI

Domène, France

news and views



Our picture of the organisation of genes in higher organisms has recently undergone a revolution. Analyses of eukaryotic genes in many laboratories¹⁻¹⁰, studies of globin, ovalbumin, immunoglobulin, SV40 and polyoma, suggest that in general the coding sequences on DNA, the regions that will ultimately be translated into amino acid sequence, are not continuous but are interrupted by 'silent' DNA. Even for genes with no protein product such as the tRNA genes of yeast and the rRNA genes in *Drosophila*, and also for viral messages from adenovirus, Rous sarcoma virus and murine leukaemia virus, the primary RNA transcript contains internal regions that are excised during maturation, the final tRNA or messenger being a spliced product.

The notion of the cistron, the genetic unit of function that one thought corresponded to a polypeptide chain, now must be replaced by that of a transcription unit containing regions which will be lost from the mature messenger—which I suggest we call introns (for intragenic regions)—alternating with regions which will be expressed—exons. The gene is a mosaic: expressed sequences held in a matrix of silent DNA, an intronic matrix. The introns seen so far range from 10 to 10,000 bases in length; I expect the amount of DNA in introns will turn out to be five to ten times the amount in exons.

This model immediately accommodates two aspects of the genetic structure of higher cells. Heterogeneous nuclear RNA clearly is the long transcription products out of which the much smaller ultimate messengers for expressed polypeptide sequences are spliced. The unexpected extra DNA in higher cells, the excess of DNA over that needed to code for the number of products defined genetically, now is ascribed to the introns.

What are the benefits of this intronic/exonic structure for genes? For the sake of argument let us assume that the splicing mechanism is general and independent of the specific gene or the state of the cell, reflecting simply some secondary structure in the RNA. For example, base-pairing in the messenger could generate sites which would serve as signals for enzymes, such as those that excise tRNAs from their precursors, to cut out a section. The cut would be

Walter Gilbert is American Cancer Society Professor of Molecular Biology at Harvard University.

Why genes in pieces?

from Walter Gilbert

resealed by an RNA ligase. Even if RNA processing is general, the presence of infilling sequences can speed evolutionary

Single base changes, the elementary mutational events, not only can change protein sequences by the alteration of single amino acids but now, if they occur at the boundaries of the regions to be spliced out, can change the splicing pattern, resulting in the deletion or addition of whole sequences of amino acids. During the course of evolution relatively rare single mutations can generate novel proteins much more rapidly than would be possible if no splicing occurred.

Furthermore, the splicing need not be a hundred per cent efficient; changes in sequence can alter the process so that base pairing and splicing occurs only some of the time. Even mutations in silent third base positions, could modify the joining so that the products of a single transcription unit can be both the original gene product and a new product, also synthesised at a high rate. Evolution can seek new solutions without destroying the old. A classic problem is resolved: the genetic material does not have to duplicate to provide a second copy of an essential gene in order to mutate to a new function. Rather than a special duplication, the extra material is scattered in the genome, to be called into action at any time. After a new gene function appears, if a higher level of product is needed, there will be selective pressure for gene duplication (as well as pressure for the loss of the introns in highly repeated genes). One consequence of the intronic model is that the dogma of one gene, one polypeptide chain disappears.

A gene, a contiguous region of DNA, now corresponds to one transcription unit, but that transcription unit can correspond to many polypeptide chains, of related or differing functions.

Recombination now becomes more rapid. Since the gene is spread out over a larger region of DNA, recombination, which should be hampered in higher cells by the inability of DNA molecules to get together, will be enhanced. Furthermore, if exonic regions correspond to functions put together by splicing to form special combinations in the finished protein, then recombination within introns will assort these functions independently. Middle repetitive sequences within introns may create hot spots for recombination to rearrange the exonic sequences.

Recombination within introns will generate curious genetic structures for eukaryotic genes. Structural mutations should be clustered, separated by long distances from mutations in other exons. Mutations in different functions may be interspersed, when one product's intron becomes another's exon.

According to this view, introns are both frozen remnants of history and as the sites of future evolution. Nevertheless, they could also have other roles. Specific recombinations between introns can bring together exons into a transcription unit to make special differentiation products. Specific new splicing patterns could be turned on by special gene products. A differentiation pathway may be determined by the appearance of a new splicing enzyme, calling forth new proteins out of the heterogeneous nuclear RNA.

On this can be based a striking hypothesis to explain the behaviour of immunoglobulin heavy chains. At an early stage of the immune response a single lymphocyte can synthesise two different immunoglobulins, IgM and IgD, with the same idiotype; two different constant portions attached to the same V_H region. This may be the result of a V_H region translocating by recombination within an intron near the constant genes so that a transcription unit is formed for a $V_H-C_H-C_\delta$ message. Splicing can then create contiguous messenger sequences for V_H-C_H and V_H-C_δ chains. The switch from IgM to IgG might be a new translocation of the V_H gene, but, alternatively, it may be a new enzyme that changes the processing of a $V_H-C_H-C_\delta-C_\gamma$ message to produce a V_H-C_γ product. □

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Restriction endonucleases: a new role *in vivo*?

from Richard J. Roberts

FEW enzymes have been exploited as thoroughly as the bacterial restriction enzymes. In recent years they have been instrumental in dramatic advances in DNA sequence analysis, genetic engineering, and studies of gene structure and function. Yet surprisingly little is known of their biological role. It has become almost axiomatic to associate them with a defence mechanism by which bacteria insulate themselves against invasion by unwelcome foreign DNA. This view has its origins in the genetic phenomenon of host-controlled restriction and modification. Two enzymes mediate this process: an endodeoxyribonuclease (restriction enzyme) which degrades DNA, and a methylase (modification enzyme) which protects DNA against the action of the restriction enzyme. Because both enzymes coexist in the same cell, the cell's own DNA is protected by the action of the methylase, whereas incoming foreign DNA, which lacks the proper modification, is susceptible to destruction by the restriction enzyme.

Two different types of restriction enzymes have been found and can be distinguished by their mode of cleavage. Although both recognise a specific sequence within a DNA molecule, the Type I enzymes cleave at random sites remote from that sequence and give heterogeneous products, while the Type II enzymes cleave at specific sites within or close to the recognition sequence. It is these latter enzymes that have enjoyed such popularity as the molecular scalpels of the biochemist. Perhaps the best-known example is *EcoRI* from *Escherichia coli* which recognises the sequence, G^+AATTC , and cleaves at the site indicated by the arrow. It produces specific fragments which carry short, single-stranded extensions at each end allowing them to reanneal and become a substrate for a DNA ligase. Thus, any two *EcoRI* fragments can be joined, which has made possible the powerful techniques of genetic engineering.

But paradoxically, the very enzymes which are thought to prevent the exchange of DNA *in vivo* are precisely those which facilitate that exchange *in vitro*. Could it be that the restriction enzymes have a dual role *in vivo* and catalyse both degradation and synthesis just as they do *in vitro*? This possibility has been raised previously but until now it had not received serious attention. It seemed unlikely that bacteria could be as smart as molecular biologists, but apparently they are. For recent

experiments by Chang and Cohen (*Proc. natn. Acad. Sci. U.S.A.* 74, 4811; 1977) show that an *E. coli* strain carrying the *EcoRI* restriction enzyme can indeed mediate genetic engineering *in vivo*. In a series of elegant experiments, they have demonstrated that a variety of site-specific recombination events can be catalysed *in vivo*.

Their experiments centred around the fact that a chloramphenicol (Cm) resistance gene contains an *EcoRI* site located so that cleavage will inactivate the gene. Restoration of gene activity requires precise rejoining at the *EcoRI* site. It was first shown that such precise rejoining could be accomplished *in vivo* by transfecting *E. coli* with two *EcoRI* fragments, each containing a part of the Cm resistance gene. Chloramphenicol-resistant transformants were recovered. Then a plasmid, pSC352, was constructed in which the same Cm gene was inactivated by the insertion of an *EcoRI* fragment. Transfection of pSC352 into an *E. coli* strain (C600, pMB4) which contained the *EcoRI* restriction enzyme resulted in a few transformants (6×10^{-5} of all transformants) which had acquired Cm resistance. *In vitro* analysis showed that such transformants arose from precise excision of the inserted *EcoRI* fragment. In more extensive experiments, they showed that *EcoRI* promoted site-specific recombination could involve multiple and physically separate fragments of plasmid DNA. In each case, the plasmids used for transformation were prepared so as to be unmodified against the action of the *EcoRI* restriction enzyme. What if the sites are already modified? To answer this question, Chang and Cohen propagated pSC352 in *E. coli* C600 (pMB4), where it is modified by the *EcoRI* methylase, and then observed the frequency of excision of the inserted fragment under normal growth conditions. Chloramphenicol-resistant clones developed with a frequency of about 10^{-9} . In this case, therefore, even the presence of the correct modification enzyme within the cell was insufficient to prevent site-specific recombination.

Although the observed frequency of these events *in vivo* is extremely low, their importance should not be underestimated. They raise anew the question of the biological role of restriction enzymes, and have implications for the current heated controversy about *in vitro* recombinant DNA experiments. Because these observations were made in the laboratory, it is difficult to assess their relevance to processes which might occur in the natural environment. Nevertheless, even if they occur at the low frequency observed by Chang and Cohen, the

magnitude of the bacterial population on the face of this Earth would still mean that the phenomenon could be of considerable importance to bacterial evolution. Such a possibility has been raised previously (Reanney *Bact. Rev.* 40, 552; 1976). Clearly, it is necessary that experiments be carried out under conditions resembling those *in vivo* for if indeed bacteria routinely exchange genetic information in this way, such exchange would not be limited to DNA originating from prokaryotes. We would be forced to the conclusion that species barriers exist, not because of a physical barrier to the exchange of genetic information, but rather because a functional barrier prevails. If such is the case, it would surely provide the strongest argument against the strict regulation of recombinant DNA experiments *in vitro*.

This question of frequency has other profound implications. If the frequency can be raised in the laboratory, then it may be possible to construct recombinant DNAs in this way. Shotgun experiments might merely require transformation of the appropriate strain with intact DNA. In this way, both the biochemistry and the present regulations could be circumvented. Of course, ethical questions remain and, presumably, the next round of guidelines for recombinant DNA research will take account of these new observations. It will be of some interest to see how they are phrased. Perhaps all experiments involving recombination, whether *in vivo* or *in vitro*, will fall under their jurisdiction; this could include the whole of classical genetics. Even the most ardent bureaucrats will find it impossible to regulate recombination in the environment. Plants, insects, animals, do it all the time. The line may have to be drawn in the laboratory. There sex will require a Memorandum of Understanding and Agreement, while elsewhere it remains unrestrained. □



A hundred years ago

THE New York *Tribune* gives an account of a public exhibition in that city of Edison's Phonograph, which seems to have been very successful. The tones reproduced by the vibrating disk of the machine were so distinct that they could be heard and understood in different portions of the crowded room.

From *Nature* 17, Feb. 7, 291; 1878.

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A RELIABLE method of assessing the mutagenicity of chemical and physical agents in mammalian cells would be invaluable. Visible chromosome damage such as breaks, and translocations has been used, but has proved rather disappointing so far; it is not as sensitive as could be wished, and while it can be effective for heavy doses of some mutagens, such as X rays, it often yields unsatisfactory results in terms of normal dose rates of ingested substances (as seen for instance in the confused literature on chromosomal breakage and LSD). Perry and Evans (*Nature* **258**, 121; 1975) showed that the number of sister chromatid exchanges (SCEs) per cell is a far more sensitive indicator of exposure to both physical and chemical mutagens than is the number of visible chromosomal aberrations, but it has not yet been proven that the presence of increased numbers of SCEs necessarily indicates actual damage to the genetic information of the cell. But now Carrano *et al.* (page 551 of this issue) have provided stronger evidence that the number of SCEs seen in cells after treatment with a range of known mutagens may be directly related to genetic damage and mutation rate, in which case it could represent a sensitive and easily quantified test of mutagenicity.

Sister chromatid exchange can be seen in chromosomes studied at the metaphase stage of cell division. At this stage each chromosome is made up of two equal chromatids which arise when the chromosomal DNA is replicated at the synthetic (S) phase of the cell cycle, a few hours before cell division starts. If material has been exchanged between these two sister chromatids by the metaphase stage it means that DNA breakage and repair must have

Testing for mutagenicity

from E. H. R. Ford

taken place between S phase and metaphase. Precise interchange of material need not of itself alter the genetic information of a cell, so the significance of visible SCE as a measure of possible genetic damage or mutation has not so far been clear.

Although SCE was described by J. H. Taylor *et al.* 20 years ago (*Proc. natn Acad. Sci. U.S.A.* **43**, 122; 1957), using an autoradiographic technique, it is only since S. A. Latt (*Proc. natn Acad. Sci. U.S.A.* **70**, 3395; 1973) introduced an optical method for differentiating sister chromatids that SCE has readily been studied. A newly replicated chromatid which has incorporated the thymidine analogue BUdR can be differentiated from its older sister by fluorescent, Giemsa staining or immunological techniques (since the BUdR quenches the fluorescent or staining property of the chromatin).

The baseline SCE frequency in untreated human lymphocytes is 5–15 per cell (Latt & Juergens in *Population Cytogenetics* (Eds Hook, E. B. & Porter, I. H., Academic Press, 237; 1977). Alkylating agents such as ethylmethanesulphonate, mitomycin C or nitrogen mustard (which can all also induce visible chromosomal damage) considerably increase the number of SCEs per cell at concentrations where no other visible morphological damage is caused to chromosomes, indicating that SCE number is a sensitive test for chromosome damage.

Carrano *et al.* have now shown that

in Chinese hamster ovary cells there is a strong and linear relationship between the induction of SCEs and of mutations producing resistance to 8-azaguanine (mutations mainly at a particular locus, the hypoxanthine phosphoribosyl transferase (HPRT) locus.) They used four mutagenic agents; the alkylating agents, ethylmethanesulphonate (EMS) and N-ethyl-N-nitrosourea (ENU), mitomycin C (MMC) which is also a cross-linking agent, and proflavine sulphate (PRO), which intercalates into DNA. Each agent produced increases in the SCE and mutation rates, and for each there was a linear relationship between SCE and mutation rate—but this was different for each agent. ENU was the least efficient inducer of SCEs compared with mutations, and MMC the most efficient. The authors have been able to calculate the number of mutations per cell which correspond to one SCE, ranging from 0.08 for MMC to 1.2 for ENU.

If certain extrapolations can be made, namely that the marker used gives a representative mutation rate comparable to that at other loci, that human cells behave similarly to those of the Chinese hamster and that the SCE/mutation ratio *in vitro* can be extrapolated to cells *in vivo*, it would be possible to estimate the mutagenicity of various physical and chemical agents simply by counting the number of SCEs found in human lymphocytes.

Carrano *et al.*'s findings need to be confirmed and the validity of these extrapolations determined. But if Carrano *et al.*'s suggestions are confirmed we may soon have a greatly improved, simple and direct method of assessing the mutagenicity of all sorts of agents.

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Preplanetary disk?

from Andrew Fabian

THE formation of stars is shrouded by dust. The massive gas clouds within which they form by gravitational collapse are cold and seeded with dust grains. Optical radiation from the newborn star is scattered and absorbed by these dust clouds, then reradiated at infrared wavelengths. Consequently it is often easier to deduce more about the properties of the gas and dust than the star. This means that little is known observationally about this important phase of a star's life.

From a theoretical point of view the situation is somewhat happier, at least until the observations improve. The collapse and fragmentation of a cold gas cloud under gravity has been discussed for decades. Problems emerge,

not the least of which are to do with the rotation and magnetisation of the star. Perfectly spherically symmetrical collapse seems most unlikely, especially when it is recalled that stars are observed to rotate at speeds up to and approaching breakup speed. Many stars are, moreover, members of binary (or multiple) pairs in orbit about each other. A collapsing rotating gas cloud is likely to flatten out and form a disk with a bulge in the centre—not too dissimilar in shape to our Galaxy. The bulge will form the stars and the disk what? Planets perhaps?—A tempting possibility considering the disk-like concentration of the major and many of the minor bodies of the Solar System. Gravitational collapse is halted within the central star, at least for the time being, by the onset of nuclear fusion, but accretion will continue within the disk until it is dispersed. Turbulent or magnetic viscosity in a disk as dense as might be associated with a newly

forming star causes matter to spiral in at the expense of angular momentum, which flows outward. We have our friend the accretion disk, which has been much discussed in the context of accretion onto black holes, both in X-ray binaries and galactic nuclei. A start was made on modelling accretion disks around newly formed stars a few years ago by D. Lynden-Bell and J. Pringle (*Mon. Not. Roy. Astr. Soc.* **168**, 603; 1974). Clearly, observational evidence of such a disk around a newly formed star is of great interest.

R. I. Thompson, P. A. Strittmatter, E. F. Erickson, F. C. Witteborn and D. W. Strecker have made a good start (*Astrophys. J.* **218**, 170; 1977) by combining their own infrared spectra of some highly reddened emission-line objects with unpublished optical continuum data of one of the objects taken by S. Grandi. MWC 349 and LK H α 107 are objects from the Mount Wilson, and Lick, catalogues of emis-

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sion-line objects. Both objects appear to be newly formed stars embedded in dense gas and dust clouds. The ultraviolet radiation of such bright young stars rapidly photoionises the surrounding gas and is thereby completely absorbed. Recombination within the resulting HII region is then responsible for the bulk of the observed emission lines. Reversing this reasoning, we see that a study of the strengths of such lines ought then to be a monitor of the ultraviolet flux of the star. Such a technique has been employed for some time, but in the case of these highly obscured objects, the emission lines must be dereddened before any reliable estimate may be made.

This is where the combination of aircraft and ground-based infrared spectroscopy comes in, for the Paschen and Brackett lines of hydrogen are much less affected by the dust reddening than the optical Balmer lines. A comparison of observed line ratios with those predicted on the basis of recombination within an HII region allows an extinction curve to be obtained. The authors use the strength of Brackett γ to estimate the emission measure of the HII region and thence (with a distance estimate) the spectral type and properties of the central star. In the case of MWC 349, a 30 solar mass 06.5 star is implied. Whilst other emission lines and radio data are consistent with this result, the observed visual magnitude is not. The inferred star is about 11 times too faint to account for the observations when reddening (using the enormous dereddening factors deduced above) is included. This might be due to a mistake in the reddening corrections, but there are problems, too, with the known 40-year brightness decrease of this object, and the flatness of its optical continuum, both of which are uncharacteristic of isolated stars.

The authors thus invoke another component—a preplanetary disk which is accreting onto the central star. It is easy to show that the long wavelength spectrum of such a disk is flatter than that of a star if both radiate as blackbodies. The inner regions of the disk are hottest, but have a smaller area than the cooler outer regions and the balance gives a spectral slope of 1/3, in agreement with the optical data. At ultraviolet wavelengths the stellar radiation dominates the ionisation of the HII region, but the disk is assumed dominant in the optical. An accretion rate is thereby deduced which together with the 40-year variability timescale suggests a disk mass at least 1% that of the Sun.

Whilst the evidence does not decisively indicate an accretion disk, or demonstrate that planets would or could form in such a disk, it does seem that the authors are justified in claim-

ing that "MWC 349 is certainly by far the best now known candidate for a

newly formed star with a surrounding preplanetary disk." □

Nations and numbers 1977

from Robert M. May

EACH year, the Environmental Fund (1302 Eighteenth St, Washington, DC) pulls together UN and other estimates, to produce a large chart of population data for individual countries, and for regional groupings. In addition to population magnitudes, and birth, death and net growth rates, the chart gives a changing selection of other statistical information pertaining to aspects of the human condition. In 1975 this included country-by-country patterns of age structure and urbanisation (see *News and Views* 261, 12; 1976), and in 1976 the number of acres of arable land per person, and the past and present figures for import or export of cereal grain (*News and Views* 265, 101; 1977).

All these numbers are uncertain. In the past, I have indicated some of the reasons, for countries as different as the USA and China. There is a major discontinuity between the Environmental Fund's world figures for 1976 and for 1977, which stems from a revision in the Indian population data. Previous numbers had been based on the 1974 population, as estimated by the International Statistical Program Center (ISPC) of the US Bureau of the Census; increasing this by the reported population growth rate gives around 657 million Indians in 1977. ISPC and the UN now base their estimates on the 1973 Indian census, giving a new figure of 642 million in 1977. This at a stroke erases 15 million bodies from the estimated world population. As the Environmental Fund notes, it also "indicates that either one or two Indian censuses were grossly in error or that Indian vital statistics are vastly worse than had been generally supposed". In eight other countries (Guinea, Ivory Coast, Senegal, Madagascar, Cameroon,

Lesotho, Saudi Arabia, United Arab Emirates) the results of new national censuses indicate population sizes greater than had been thought.

Overall, the world population in mid-1977 was around 4,307 million, growing at an average rate of 2.1% per annum. Highest growth rate was in Libya, with 4.1% per annum; Kuwait's 6.1% was disqualified, as influenced by immigration. Lowest was Portugal, with -0.4%. Many of the local differences in average population growth rates (for example 3.6% in Gaza versus 2.1% in Israel) have political consequences.

Regional patterns over the sweep of the past 27 years are interesting. Since mid-1950, the world population has grown from around 2,543 to 4,307 million, representing an average growth rate of 2.0% per annum over that period (as contrasted to the 1977 figure of 2.1%). The regional components are as set out in the table.

Another pattern documented in the 1977 Environmental Fund chart concerns literacy rates. These rates for individual countries have been collected from various sources (UNESCO, US Department of State, CIA and others), and they are likely to be vagariously defined and of questionable accuracy. Even so, it is interesting to plot the literacy rates against the population growth rates for the 157 countries included in the chart. This is done in Fig. 1. The black squares are regional averages (Africa, 22% literacy and 2.8% population growth rate; Asia, 49 and 2.4; North America, 99 and 0.8; Latin America, 65 and 2.8; Europe, 96 and 0.6; USSR, 98 and 1.0; Oceania, 84 and 1.8), and the large star is the global average (59% literacy and 2.1% growth rate).

No simple message emerges from Fig. 1. It does suggest that low population growth (below 1% say) is correlated with high literacy, but even here Portugal

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Region	Population in mid-1950 (millions)	Population in mid-1977 (millions)	Average growth rate (% p.a.)	1977 growth rate (% p.a.)
Africa	219	451	2.7	2.8
Asia	1,409	2,506	2.1	2.4
North America	166	247	1.5	0.8
Latin America	164	342	2.7	2.8
Europe	392	479	0.7	0.6
USSR	180	259	1.3	1.0
Oceania	12	22	2.2	1.8

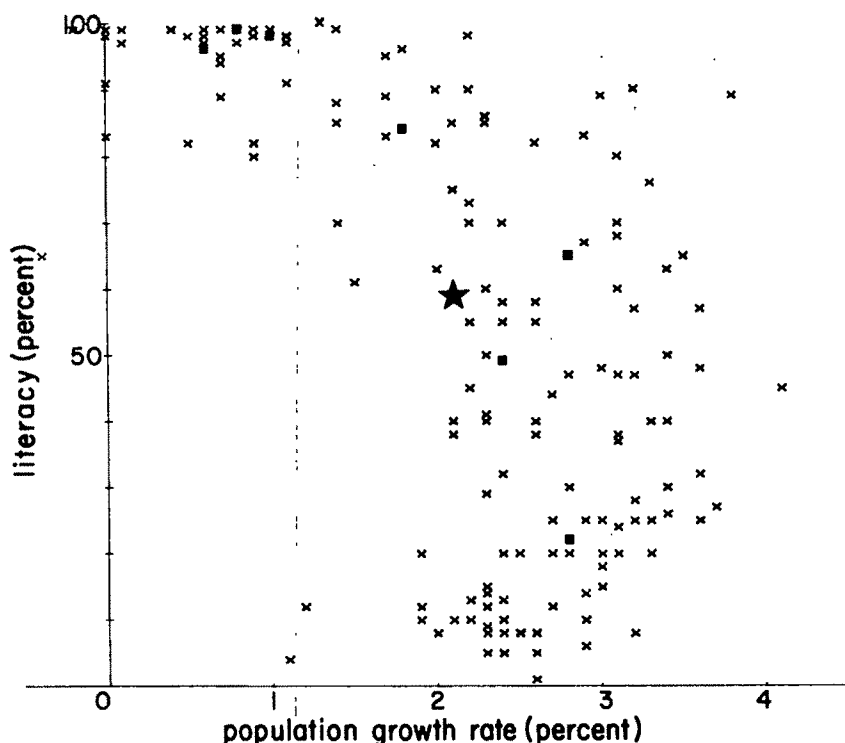


Fig. 1 Percentage literacy is plotted against annual population growth rate for 157 countries (a few of the crosses represent two countries). The solid squares are regional averages, as described in the text; the large star is the global average.

(-0.4% population growth with 65% literacy) is an exception. At the other end, although there are a lot of countries with population growth rates in the range

2-4% and literacy rates below 30%, the figure does not support any unambiguous correlation between high population growth and low literacy. □

New ways to look at X inactivation

from M. Bobrow

MAMMALIAN embryogenesis is very sensitive to alterations in the 'balance' of genetic material. Additions or losses of whole chromosomes usually produce severe phenotypic derangements. In this context, the sex chromosomes are somewhat of an anomaly. The Y chromosome contains little if any genetic information other than that directly relevant to inducing testicular differentiation of the primitive gonad. The X chromosome is large and contains at least several hundred gene loci having nothing to do with sex differentiation. However, the female develops normally with two X chromosomes, while the male manages at least as well on only one.

The crucial mechanism behind this phenomenon is a regulatory system which allows only one X chromosome to remain active in a diploid cell. First proposed in 1961 (Lyon *Nature* **190**, 372) the 'Lyon hypothesis' has been proven

substantively correct, but details of the process are still obscure (well reviewed by Gartler & Andina *Adv. hum. Genet.* **7**, 99; 1976).

The basic hypothesis states that, at some stage during early embryogenesis, all X chromosomes bar one are inactivated and are no longer available for transcription. Which X remains active in any given cell is randomly determined. The process involves a whole chromosome rather than individual genes, and is accompanied by physical changes in the chromosome—it is visible as a highly condensed body during interphase (the Barr body) and the timing of its DNA synthesis is shifted to a later stage of the cell cycle than its active homologue. Whatever the nature of the inactivation process it is heritable, so that descendants of a given cell form a clone. Every normal female is thus a somatic cell mosaic, some cells having an active paternally-derived X, and others an active maternal X. The growth of knowledge concerning biochemical markers of X chromosome activity, and the powerful new techniques for manipulating the genetic constitution

of mouse embryos (by forming chimaeras, for example), has led to a recent wave of interest in the timing, extent and randomness of X inactivation.

Cytological evidence of X inactivation first appears in the early blastula of the mouse (± 40 cell stage). Functional inactivation was studied in a most elegant experiment: single cells from 3.5-4.5 d blastocysts were injected into host blastocysts, and formed chimaeric mice displaying patches of activity of both X chromosomes of the injected cell—implying that cell transfer had preceded X inactivation. (Gardner & Lyon *Nature* **231**, 385; 1971) Studies of 'patch size' of X-linked markers in adult female heterozygotes lead to rough estimates of 10-60 cells present in the embryo at the time of X inactivation.

Another approach to the timing of X inactivation is the biochemical analysis of X-linked gene products in the early embryo. Before inactivation, XX embryos should produce twice as much gene product as their brothers, which would lead to a bimodal distribution of gene activity. An obvious pitfall is that unimodal distributions of enzyme activity in very early embryos may reflect pre-fertilisation oocyte transcription.

A recent example of this approach (*Nature* **270**, 599; 1977) is the study of Monk and Kathuria, who analysed HGPRT activity in single mouse embryos. They found a unimodal distribution of activity in 8-cell embryos and in pre-implantation blastocysts—at which stage the embryonic genome is certainly active. There is evidence of embryonic synthesis of HGPRT from a very early stage of embryogenesis, and this finding would therefore suggest that X inactivation has occurred well before implantation; earlier than suggested by the other approaches. This discrepancy could be due to methodological difficulties in one or more of the techniques; inactivation occurring at different times in different embryonic cells; or to inactivation not affecting the whole chromosome simultaneously. An intriguing speculation would be that regions of the chromosome are inactivated as their transcriptional products are required in the differentiating embryo.

The concept of random inactivation seems to be generally valid, but three major exceptions have been discovered. X/autosome translocations and structurally abnormal X chromosomes in both man and mouse show preferential inactivation of one or other X chromosome—but these situations have only been studied in adult animals and may represent post-inactivation cell selection. Information on inactivation in early embryos with X/autosome translocations would be most interesting. In marsupials, the paternal X chromosome is preferentially inactivated and a similar situation pertains in the extra-embryonic membranes of rodents (refs in Gartler &

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Andina *op. cit.*). This seems to imply some form of chromosome 'imprinting' to mark the paternal X chromosome, and it has been suggested that this is a fundamental and primitive form of X inactivation, from which some cells of the eutherian embryo have learned to escape. That imprinting is not a necessary prerequisite of X inactivation has been finally demonstrated by Kaufman, Guc-Cubriilo and Lyon (this issue of *Nature*, page 547) who have provided cytological evidence of X inactivation in the absence of any paternal genetic contribution, using parthenogenetic embryos created by suppression of polar body formation.

Despite a variety of interesting suggestions on mechanisms for permanently modifying DNA bases or structural configuration (see refs in Gartler &

Andina *op. cit.*) virtually nothing is known of the biochemical or biophysical basis of X inactivation. This, and many other aspects of this fundamental process of mammalian genetic regulation, may become amenable to investigation following the exciting discovery of Martin *et al.* (*Nature*, 271, 329; 1978). These workers have demonstrated that mouse teratocarcinoma cell lines maintained *in vitro* show biochemical evidence of X inactivation if they are grown under conditions which allow them to initiate morphological differentiation. If this experimental model turns out to be as useful as it promises on first sight, our understanding of the process of X inactivation, and of whole-chromosome genetic regulatory mechanisms, may advance rapidly over the next few years. □

Smoothing primaeval chaos

from Paul Davies

THERE are two conflicting philosophies about the nature of the Universe. The first, which meshes well with traditional religious beliefs, is that the world we live in is a very special sort of place, elaborately constructed to be just the way it is and no other. The second holds that there is nothing really remarkable about the Universe, given the laws of physics. According to the latter, the world is a pretty random sort of place. The nexus of this controversy focuses on the creation event—the so-called big bang—which apparently took place about 15 billion years ago and represents the origin of all space, time and matter. Assuming the reality of this universal genesis, the question naturally arises as to the precise condition in which the Universe was created. Some cosmologists contend that it began in a highly special and extremely improbable condition, others that it started out as though God had chosen the world randomly from all possible alternatives.

To defend the second philosophy, it is necessary to demonstrate that there exists a very wide range of initial conditions that would cause the Universe to subsequently evolve into something closely similar to what we observe today. Another way of expressing this is to say that our basic world structure is stable against random changes in its original state: that almost any sort of big bang would have led inevitably to a Universe of the type we have.

In the past few years a lively debate

has arisen around this question, as our understanding of the primaeval cosmos improves, both through astronomical discovery and theoretical modelling. The central observational fact is that, on an extragalactic scale, the Universe appears to be very uniform and regular, both as regards the distribution of matter in space and the systematic pattern of galactic recession which reveals the overall expansion of the cosmos. The great mystery is: why is the Universe so orderly? Was it simply created in this remarkably uniform condition, or has it contrived to evolve order out of primaeval chaos? A further twist to the problem is that without some degree of irregularity the galaxies themselves would not have formed. Galaxies arose because certain regions of the primaeval gases erupting from the big bang were slightly denser than others, and the locally enhanced gravity associated with this clumping proceeded to trap the surrounding material and prevent it from dispersing under the action of the universal cosmological expansion. In this way, initially rather tenuous irregularities inexorably grew in size and contrast until they became the isolated entities that we now observe.

Several years ago intensive investigations began into the fate of a cosmos which starts out with a large amount of turbulence. Several mechanisms exist which would operate to smooth out the primaeval turbulence. For example, fluid viscosity can damp away chaotic motions and restore orderly flow in many everyday systems. In a cosmological context, the viscosity of

neutrinos (elusive subatomic particles) was suggested by the American cosmologist Charles Misner as a cause of viscous damping operating at about 1 second after the big bang, when the Universe had a temperature of 10 billion degrees. Other damping processes have since been discussed; quantum particle production around 10^{-23} s, graviton collisions at 10^{-20} s, hadron collisions at 10^{-8} s, microscopic black hole production, and so on. All these processes operate to reduce the turbulent chaos and establish a more orderly and regular arrangement of matter.

On the face of it, one could well suppose that, through these many mechanisms, even a highly irregular primaeval cosmos would soon convert into something like the present condition. The snag is that, according to the second law of thermodynamics, the establishment of order out of chaos has to be paid for by an increase in total entropy, most of which appears as heat energy. The heat of the primaeval Universe can still be detected as the celebrated cosmic background thermal radiation, which provides us with a measure of the entropy of the Universe. Although the temperature of this radiation is now very low (about 3 K), it still represents a vast amount of entropy per atom of matter.

In a recent paper (*Mon. Not. R. Astr. Soc.* 181, 719; 1977) John Barrow of Oxford University and Richard Matzner of the University of Texas at Austin have re-examined the relationship between the cosmic entropy and the primaeval turbulence. They point out that although the entropy is large (indicating that a definite amount of dissipation occurred at some stage in the past) it is obviously still finite, and can be used to provide an upper limit on the amount of primaeval disorder. They argue as follows: observations of the thermal background radiation and studies of nuclear processes in the primaeval matter indicate that the Universe was highly isotropic (regular in all directions) as early as a few minutes after the creation event, so any initial anisotropy must have been dissipated before that time, and converted into heat radiation. Now the energy of heat radiation is greater at earlier moments, as one probes closer to the initial creation event, because the Universe is more compressed and so the temperature is higher. Similarly, the energy of the anisotropy turbulence also grows as one approaches the beginning. What Barrow and Matzner point out is that the anisotropy energy grows much more rapidly than the heat energy. The crucial consequence of this observation is that the earlier the anisotropy was dissipated, the

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greater the heat (that is, entropy) it would have produced. For example, if dissipation occurred as early as the so-called Planck era (10^{-43} s) when quantum gravity effects were important, then even a minute amount of initial anisotropy would have produced too much entropy.

Without a detailed understanding of the dissipative mechanisms in the very early stages, it is impossible to place precise numerical constraints on the permitted degree of primaeval turbulence, but the authors conclude on general grounds that an initially highly chaotic cosmology can be ruled out. There is still the problem of accounting for the existence of galaxies in this quieter scenario, a phenomenon about which only rudimentary understanding has been gained.

If Barrow and Matzner's ideas continue to hold for more elaborate models of the big bang, it seems that we shall inevitably be obliged to face the mystery that the Universe has been created in a remarkably orderly and highly special state after all. One possible approach to this mystery is through the anthropocentric—that if the cosmos were not so regular we could not be here to know about it. A highly turbulent big bang would overproduce entropy and the cosmic background radiation would then be too hot for life to evolve until it had cooled below, say, the boiling point of water. But the temperature of this radiation takes billions of years to diminish appreciably, and if it were substantially hotter than now then the stars would have burnt out before conditions became cool enough for life to form. Without stars such as the Sun there would be no life anyway. This is not, of course, an explanation for the orderly Universe, but it provides an intriguing comment on how lucky we are that the big bang was so well-behaved. □

Red deer or takahe?

from Peter D. Moore

THE conservation of a species normally demands an active management of its habitat in order to increase its carrying capacity for that particular species. Where a species requires a habitat in its early stages of succession, this may mean extensive and frequent modifications of the habitat to prevent its maturation. Generally the amount of management effort declines in situations requiring more advanced successional stages, and for climax ecosystems the major problems are often ones of

prevention of destruction of the habitat, or the control of exotic, invasive predatory or competitor organisms. Experimentation, based upon sound and detailed ecological survey and rational extrapolation, is often necessary before the best available management techniques can be devised and implemented.

Such an experimental approach to conservation is most hazardous where one is dealing with an organism on the brink of extinction. Here one cannot afford to make mistakes. Take, for example, the takahe (*Notornis mantelli*), a flightless gallinule endemic to New Zealand. Subfossil remains of the bird suggest that it once had a fairly extensive range in the south-western tip of the South Island of New Zealand, but the European settlers of the nineteenth century found few specimens and during the first part of this century it was considered extinct. Then, in 1948, it was dramatically rediscovered in the high, alpine and sub-alpine tussock grassland of the Murchison Mountains. It is now known to occur in an area of less than 1,000 km² in these mountains and its population in some parts of this area may still be declining.

Obviously, in such a situation, considerable information is required concerning the environmental requirements of the takahe, before management can be applied. One adverse influence in the area may be the introduced red deer (*Cervus elephas*). Questions such as whether these two species are in competition and whether the deer are modifying the habitat in a manner unfavourable to the takahe, need to be answered.

Mills and Mark (*J. Anim. Ecol.* 46, 939; 1977) have attempted to provide the information needed to answer these questions. They studied a series of 10 m × 5 m plots in tussock grassland within takahe territory which were situated among the four major dominant tussock-forming plants, *Chionochloa pallens*, *C. flavescens*, *C. teretifolia* and *C. crassiuscula*. Usage of the plots was assessed by counting the droppings within them, which can be expected to reflect the amount of time spent by takahe in each. Damage to plants (the bird is herbivorous) could also be estimated directly since tussock tillers are pulled off, the base eaten and the remainder discarded in a pile.

This type of study has led Mills and Mark to conclude that the preferred food of the takahe consists of *C. pallens* and *C. flavescens*, their relative proportions varying seasonally. Plots dominated by these two species were

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also richer in deer faeces, particularly *C. flavescens*. Chemical analyses of the tillers of four *Chionochloa* species showed that the preferred two species exceeded the others in their concentration of N, P, K, Ca, Mg and soluble sugars (P and K statistically significant). This was not the case for S, Na and starch content. *C. teretifolia* was the species with the lowest overall food value and this species was not recorded as eaten by takahe in the study plots.

From this study it is evident that the two species, *C. pallens* and *C. flavescens*, are more nutritious and are preferred as food sources by the takahe. The red deer, however, has similar food preferences and this fact, together with the limited abundance of the two plant species (20% and 10% cover respectively) indicates that competition for food may be taking place between the two species. Analyses of individual tussocks which had been selected for grazing suggest that both takahe and red deer selected plants rich in phosphorus for their attention. Overall, the red deer had a more varied diet, including more dicot species, but the coincidence of their preference for phosphorus-rich *C. flavescens* tussocks confirms an overlap of niche and hence a competitive interaction. Severe competition, however, may be avoided by the fact that deer take the leaf blades whereas takahe take the basal sheaths.

The impact of the competitive interaction is likely to be most severe when some other factor, such as prolonged snow cover in a severe winter, causes harder grazing by the deer and consequent damage to the structure and composition of the alpine grasslands. Under such conditions, of course, the first plants to suffer will be those with the highest phosphorus content, thus depriving the takahe of their nutrient resource.

The New Zealand Forest Service is currently conducting deer population control operations in the area and the work of Mills and Mark suggests that this could have beneficial effects not only for forestry but also for the alpine grasslands and the takahe. For this endangered species, the removal of red deer is likely to do nothing but good.

Order in amorphous polymers

from Paul Calvert

THE problem of whether there is long range order in liquid and glassy polymers has long been troubling polymer science. The 1975 Shrivernham Meeting on Polymer Physics followed closely after a symposium on 'Physical struc-

ture of the amorphous solid state' (*J. macromolec. Sci.* **B12**, 1; 1976) and it seemed that the question was settled; there was no long range order. By the 1977 Shrivenham Meeting the problem had returned, as intractable as ever.

The description of order in liquids and glasses has always been difficult. Bernal's model of dense random packing of liquids was derived by filling a balloon with ball bearings, kicking it round the laboratory, then fixing the structure by pouring in paint so that it could be disassembled and analysed. Regrettably a similar experiment for chain molecules has not been done.

Electron micrographs of thin films of amorphous polymers show 20–50 Å nodules which G. S. Yeh (*J. macromolec. Sci.* **B6**, 451, 465; 1972) claimed to be due to locally ordered regions surrounded by less ordered material. Many microscopists believe these nodules to be artefacts of beam damage, and at the 1975 symposium this straw man was soundly beaten. Small angle X-ray scattering showed unambiguously that there are no significant density fluctuations and small angle neutron scattering demonstrated that the molecules had the same overall radius of gyration as in θ (ideal)-solution suggesting that there could be no ordering. However, semicrystalline polymers have since been found also to have this random coil radius of gyration, so this result must be compatible with order. Yeh has modified his ideas to suggest that the observed blobs are a diffraction effect from regions where the chains are locally parallel. Similar to this is the Pechold meander model (Pechold & Blasenbrey *Koll. Zeit.* **241**, 995; 1970) in which the structure consists of curved, randomly packed bundles of parallel chains. At Shrivenham 1977 it was shown that by folding the bundles correctly a suitable molecular radius of gyration could be produced. There is really no such explicit model of a structure with long range order.

To complement the shortage of models there is also a shortage of methods. Using X-ray diffraction data Warren and Mozzi (*J. appl. Crystallogr.* **2**, 164; 1969) produced a convincing structure for glassy silica based on misalignments of adjacent SiO_4 tetrahedra, but polymers contain more atoms per repeat unit and there is just not enough information in the few broad diffraction peaks that are observed. Wecker, Davidson and Cohen (*J. Materials Sci.* **7**, 1249; 1972) found that the structure in amorphous isotactic polystyrene closely resembled the crystalline state, implying that the chains adopted the same helical structure in each case, and the main change was in the relative arrangement of the helices. Windle and

Lovell (*Polymer* **17**, 488; 1976) have been squeezing more information out of these systems by looking at X-ray diffraction of oriented polymers (this ploy of using fibre patterns is how most crystalline polymer structures have been solved). The spacings of reflections parallel to the chain axes allow the helical conformations to be determined. However, the samples are not really sufficiently oriented, so that it is necessary to sharpen the patterns by analytical methods which might introduce spurious peaks. The results reported at Shrivenham 1977 confirm Wecker's isotactic polystyrene results, propose a predominantly ttgg (t, trans; g, gauche) helix with some tttt for commercial atactic polystyrene which is 70% syndiotactic and suggest a ttg helix for polymethylmethacrylate (Perspex). A rule due to Natta for polymer crystal structures is that intermolecular interactions do not usually distort the chain from the minimum energy conformation of an isolated chain. These results suggest that the predominant helix in amorphous polymers is that found in the crystal structure, so that a similar rule could be proposed that the distribution of conformations in liquid, glass and θ -solvent is the same as that calculated from intramolecular potentials alone. But this still says nothing about how the chains are packed together.

Markova and coworkers claim to have detected regular intermolecular spacings indicative of ordered regions of at least 50 Å by electron diffraction (*J. Polym. Sci. C* **42**, 671; 1973) though

their data analysis has been criticised (Voigt-Martin & Mijchhoff *J. appl. Phys.* **46**, 1165; 1975). At Shrivenham 1977 Longman and Wignall described the level of order in polyethylene as being similar to that in liquid paraffins. Since, however, it is impossible to find two groups in this field who accept each other's data analysis, it is hard to know how firm these conclusions are.

In principle infrared spectroscopy or NMR ought to be able to detect chain conformations associated with kinks in the helix where the chain changes direction, but I am not aware of any such quantitative measurements. Light scattering can detect regions of local orientational order, thus Brillouin scattering and depolarised Rayleigh scattering both support Longman and Wignall's conclusion that polyethylene is no more ordered than paraffin chains of 20–30 atoms (Patterson *et al. Macromolecules* **10**, 667, 737; 1977; *J. macromolec. Sci.* **B12**, 61; 1976.)

If, then, we ask whether chains in the amorphous state adopt the same conformations as in θ -solvent the answer so far seems to be yes. On this basis the length of the straight sections of chain will depend on chain stiffness, in line with the observation that stiff molecules such as polytetrafluoroethylene and poly-4-methyl pentene show particularly sharp X-ray diffraction peaks in the liquid state, implying increased order. We must now ask if it is possible to pack the molecules to the required density without them lying parallel, and so far there is really no answer to this. □

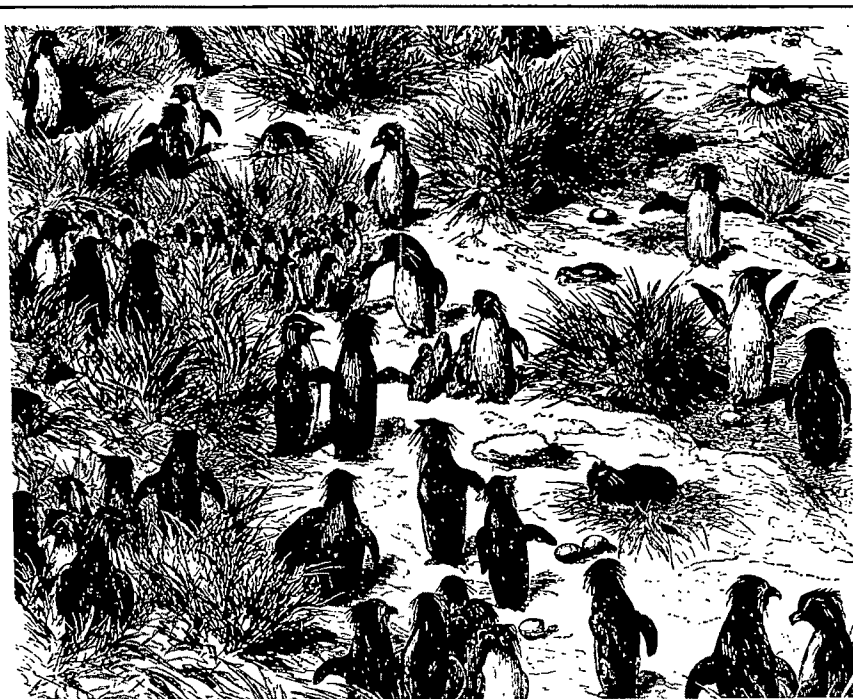


ILLUSTRATION of penguins (*Eudyptes chrosolopha*) and young on the islands of St Paul and Amsterdam. From an account of an expedition to these islands published in *Archives de Zoologie Expérimentale et Générale* (6; 1877).

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review article

Handedness of atoms and parity non-conservation

G. Feinberg*

Experiments to test the existence of parity non-conserving interactions in atoms are described. Such experiments have been done in heavy atoms, but their theoretical interpretation is difficult. Prospective experiments in hydrogen and deuterium should clarify the situation, and determine which unified gauge theory can describe weak and electromagnetic interactions.

THE phenomenon of handedness, that is, the existence of physical systems that are not identical to their mirror images, is well known for macroscopic systems, such as the human body. It is also well known that some molecules, such as tartaric acid, occur in two forms with opposite handedness, which can be distinguished from each other by their property of rotating the plane of linearly polarised light in opposite directions¹.

The existence of handed molecules has nothing to do with parity non-conservation, that is, with a failure of the complete molecular energy operator to be invariant under space reflection, although it is conceivable that the prevalence of one type in living things on earth is related to such failure². The two types of molecule are both quasi-stable states, and their existence is the consequence of near degeneracies between opposite parity energy levels of a parity conserving electrostatic potential energy³.

If the atomic potential energy contains some small terms that do not conserve parity, then it is possible that in isolated atoms, where such near degeneracies are not found, there may occur a more profound type of handedness, such that the mirror image of an atomic state is not a stable state at all. This would be analogous to the situation for neutrinos: as far as we know, only left-handed neutrinos occur in nature, and the mirror image right-handed neutrinos do not exist⁴.

If any such handedness occurs in atoms, it must be very small⁵. Furthermore, until about 1968 there was little theoretical reason to expect any parity non-conserving terms in the atomic potential energy. But over the past 10 years, the invention of theories that unify weak and electromagnetic interactions⁶, and the apparent success of such theories in describing neutrino scattering, have provided new stimuli to the search for handedness in atoms.

In these unified theories, the interaction energy between electrons and nuclei originates not only from the exchange of photons, but also from the exchange of massive neutral, spin-1 particles. This new interaction energy, unlike that coming from photon exchange, may not be invariant under space reflection, because the spin-1 Z particles that are exchanged, have as their sources both vector and axial vector currents which behave oppositely under space reflection. The potential coming from Z particle exchange is also very short range because of the high mass of the Z particle, and so involves the small probability of finding an electron at the nucleus of the atom. In terms of the electron wave functions the parity non-conserving interaction may be written as

$$H_{PV} = \frac{G_F}{\sqrt{2}} \sum_i \sum_j \left[(C_{1P} \psi_i \gamma_\mu \gamma_5 \psi_i(\gamma^\mu)_{P_j} + C_{1N} \psi_i \gamma_\mu \gamma_5 \psi_i(\gamma^\mu)_{N_j} + C_{2P} \psi_i \gamma_\mu \psi_i(\gamma^\mu \gamma^5)_{P_j} + C_{2N} \psi_i \gamma_\mu \psi_i(\gamma^\mu \gamma^5)_{N_j} \right] \quad (1)$$

In this formula ψ_i are the Dirac wave functions of the electrons in the atom, evaluated at the nucleus, and the sums i, j are carried out over the electrons and the neutrons (N) and protons (P) respectively. The constant G_F is the weak interaction coupling constant ($G_F \approx 10^{-49}$ erg cm⁻³). C_{1P}, C_{1N}, C_{2P} , and C_{2N} are numbers which vary from one unified gauge theory to another, and are the quantities that theorists would most like to find from a study of atomic handedness. In the standard theory of Weinberg and Salam^{6,7} the values of the C_i are given by

$$\begin{aligned} C_{1P} &= \frac{1}{2}(1-4\sin^2\theta) & C_{2P} &= \frac{1}{2}(1-4\sin^2\theta) \\ C_{1N} &= -\frac{1}{2} & C_{2N} &= -\frac{1}{2}(1-4\sin^2\theta) \end{aligned} \quad (2)$$

The quantity $\sin^2\theta$ must be determined from experiment. The neutrino experiments⁸ are fitted by the standard theory with $\sin^2\theta \approx 1/4$. In other unified gauge theories, more complex than the original theory, the values of these constants may be different. Indeed, in one class of theories, there may be little or no parity non-conserving interaction between electrons and nucleons. Therefore, the detection and precise measurement of atomic handedness can provide a crucial test of these forms of the gauge theory.

The terms in H_{PV} will have matrix elements between states of opposite parity. The terms involving C_1 are proportional to the number of protons (Z) and neutrons ($A-Z$) in the nucleus, while those involving C_2 are proportional to the spins of the proton and neutron. We shall see that the latter terms are less important in heavy atoms, and in experiments that are insensitive to the nuclear spin alignment. Because each term involves the electron wave functions at the nucleus, the only states for which the matrix elements are not negligibly small are $S_{1/2}$ and $P_{1/2}$ states. In other states, the Dirac wave functions all vanish at the nucleus. Accordingly, H_{PV} will lead to a parity mixing in $S_{1/2}$ and $P_{1/2}$ single electron states. That is, an $S_{1/2}$ state will actually contain a small admixture of various $P_{1/2}$ states and conversely.

The amount of this admixture, which measures the handedness of the atom, will be given, for a specific $S_{1/2}$ state, by

$$\delta(S, nP) \sim \frac{\langle S_{1/2} | H_{PV} | nP_{1/2} \rangle}{[E_{S_{1/2}} - E_{nP_{1/2}} + (i/2)(\Gamma_{S_{1/2}} - \Gamma_{nP_{1/2}})]^{-1}} \quad (3)$$

where E is the energy of the state, and Γ is its decay width.

This mixing can be calculated straightforwardly for a hydrogen or deuterium atom. It is found that in such atoms by far the largest mixing occurs between $S_{1/2}$ and $P_{1/2}$ states of the same principal quantum number, such as $2S_{1/2}$ and $2P_{1/2}$. This is because the energy difference between these states is abnormally small, given by the Lamb shift, which is of order 10^{-6} eV, compared to several eV for the energy difference between states of different principal quantum number. Because of the presence of the nuclear spin dependent terms in H_{PV} , the mixing depends on which of the

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Table 1 Calculations and experimental results on optical rotation in bismuth

Wavelength of transition (Å)	Experiment	Theory, ref. 18	Theory, ref. 21	Theory, ref. 20	Theory, ref. 16
8,757 Å	-0.07 ± 0.32 (ref. 14)	-2.3	-1.2	-2.3	-1.6
6,477 Å	$+0.27 \pm 0.47$ (ref. 15)	-3	-1.6		-1.7

The numbers given are for $10^7 \times \text{Im}M(E1)$. Most of the calculations use the value $\sin^2\theta = .35$. Use of the value $\sin^2\theta = 0.25$ will decrease the theoretical values by a factor of about 0.8.

hyperfine states in hydrogen are being mixed. In the case of zero external magnetic field, the mixing can be written as⁹

$$\delta(2S_{1/2}, 2P_{1/2}) = -i\bar{C}(1.2 \times 10^{-11}) \left(1 + \frac{i(\Gamma_{2S_{1/2}} - \Gamma_{2P_{1/2}})}{2(E_{2S_{1/2}} - E_{2P_{1/2}})}\right)^{-1} \quad (4)$$

In this equation

$$\bar{C} = C_{1P}Z + C_{1N}(A-Z) - [F(F+1) - I(I+1) - \frac{3}{4}] / (C_{2P}K_P + C_{2N}K_N) \quad (5)$$

where F is the total angular momentum and I is the nuclear angular momentum. K_P and K_N are 2 and 0 in hydrogen, and both 1 in deuterium. The ratio

$$\frac{\Gamma_{2S_{1/2}} - \Gamma_{2P_{1/2}}}{2(E_{2S_{1/2}} - E_{2P_{1/2}})}$$

is approximately 1/20 in hydrogen or deuterium.

It can be seen from equation (4) that if the C_i values are numbers of order 1, as expected in the Weinberg model from equation (2), then the mixing in neutral hydrogen or deuterium is approximately

The small value of δ is a consequence of the very short range of the weak interactions that generate H_{PV} , in comparison with the size of an atom. This range is approximately 10^{-16} cm, and the probability of finding an electron that close to a nucleon in an atom is very small indeed.

This admixture of opposite parity states will induce a small handedness for a hydrogen atom in a $2S_{1/2}$ or $2P_{1/2}$ state. Because of the very small value of δ it seems difficult to measure this handedness in hydrogen atoms without external fields. Two alternative approaches to the measurement of the atomic handedness predicted by the unified weak interaction theories have therefore been proposed.

Heavy atoms

In atoms with large nuclear charge, such as bismuth, with $Z = 83$, the parity admixture δ can become somewhat larger than in hydrogen, for two reasons. One is that the electron wave functions at the nucleus are much greater in magnitude, as even an S-state valence electron is more likely to be found near a heavy nucleus than is an electron in hydrogen. The other reason is that the terms in H_{PV} involving C_{1P} and C_{1N} are proportional to Z and $(A-Z)$, and so are 100 times or so greater than in hydrogen. On the other hand, the nuclear spin dependent terms involving C_{2P} , C_{2N} are not proportional to Z or $(A-Z)$ and so are presumably 100 times smaller in heavy atoms, and negligible as a first approximation. These enhancement effects in heavy atoms are somewhat counteracted by the fact that the energy differences of opposite parity states of valence electrons of heavy atoms are of order 1 eV, rather than the 10^{-6} eV of the $2S-2P$ splitting in hydrogen. Calculations by Bouchiat and Bouchiat¹⁰ indicate a typical value for the mixing coefficient between individual states of opposite parity in a heavy atom such as Bi, of about 10^{-10} , or 10 times greater than the $2S-2P$ mixing in hydrogen.

Two types of experiment have been attempted to detect the parity mixing in heavy atoms. One type, proposed by Bouchiat and Bouchiat¹⁰, is in progress in Paris¹¹ and at Berkeley¹². It

involves comparing the absorption rates of left-circularly polarised and right-circularly polarised photons by the heavy atoms. If the atoms have a slight handedness, these rates will be different. The difference arises because the absorption can, in the presence of handedness, occur both through magnetic dipole ($M1$) and electric dipole ($E1$) transitions, and these two terms interfere to give a difference in the absorption rates. A situation is chosen in which the $M1$ transition would occur even in the absence of parity mixing whereas the $E1$ transition only occurs because of this mixing. In this situation, the absorption rates of the two types of photons are proportional to

$$R_{\pm} \sim (M_{M1} \pm \delta M_{E1})^2 \quad (6)$$

where δ is the mixing, and M_{M1} , M_{E1} are the matrix elements for the $M1$ transition between the unmixed states, and for the $E1$ transition between the admixed state and the unmixed part of the other state. Actually δM_{E1} must be summed over all states that can be admixed into the original states.

The relative difference in the absorption rates is for small δ

$$\frac{\Delta R}{2R} \equiv \frac{R_+ - R_-}{R_+ + R_-} \sim 2 \frac{M_{E1}}{M_{M1}} \delta \quad (7)$$

Since M_{E1}/M_{M1} is usually large, this difference is much greater than δ itself, and so is capable of measurement. In the actual experiments in progress, there are additional complications involving static electric fields, which are introduced to help amplify the handedness further, and to distinguish it from background effects.

In the other type of experiments, use is made instead of a relation between the difference of absorption rates for the two circular polarisations of photons, and the angle through which the plane of polarisation of linearly polarised light at resonant frequency is rotated upon passing through a medium consisting of bismuth vapour. This relation is given

$$\Delta\theta \sim \frac{\pi N(\omega - \omega_0)}{(\omega - \omega_0)^2 + \frac{1}{4}\Gamma^2} [R_+ + R_-] \text{Re}(R_+ - R_-) \quad (8)$$

In this formula, N is the number density of Bi atoms, l the path length through the vapour, ω the light frequency ω_0 the resonant frequency, and Γ the width of the resonance. The path length l is limited by the absorption, so that l cannot be more than a few times the absorption mean free path so that

$$1/l \approx \frac{N[R_+ + R_-]^2 \Gamma 2\pi}{4[(\omega - \omega_0)^2 + \frac{1}{4}\Gamma^2]} \quad (9)$$

From equation (8) and (9) we get

$$\Delta\theta \sim \frac{\omega - \omega_0}{\Gamma/2} \frac{\text{Re}(R_+ - R_-)}{R_+ + R_-} \quad (10)$$

Therefore a measurement of $\Delta\theta$ determines the same quantity as does $\Delta R/R$ in the other type of experiment. It is apparently easier to measure a small rotation angle than to measure a small change in absorption rate, and so the optical rotation experiments have proceeded more rapidly. Two experiments of this type have already been done¹³⁻¹⁵ with results summarised in Table 1.

In order to interpret these experiments, it is necessary to

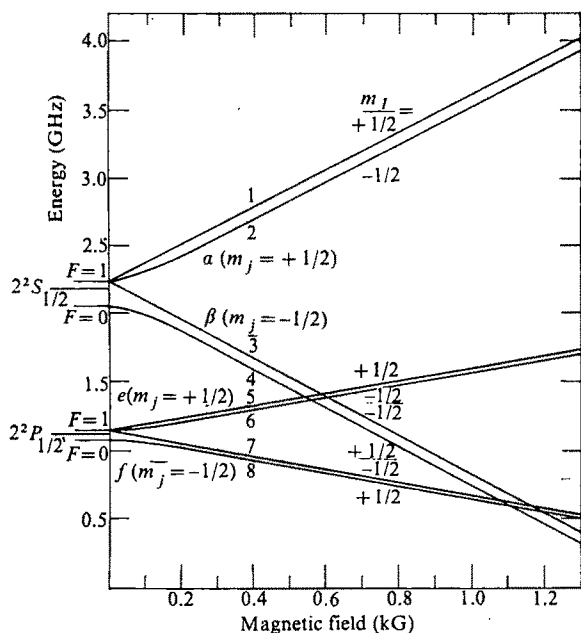


Fig. 1 The Breit-Rabi diagram for hydrogen, with $n = 2$.

calculate R_+ , R_- for the atomic transitions involved. The calculation of M_{M1} , or its direct measurement for the transitions of interest, is not difficult. On the other hand, the calculation of M_{E1} presents substantial problems, even if the values of C_{1P} and C_{1N} are specified. This is because in heavy atoms, no single state dominates the mixing as it does in hydrogen. Instead, a large number of opposite parity states have comparable energy differences from a given state, and are therefore mixed into it by H_{PV} with comparable coefficients. In order to obtain an accurate value for M_{E1} , it is necessary to sum over all such states. We can write

$$M_{E1} = \sum_n \langle i | H_{PV} | n \rangle \frac{\langle n | (E1) | f \rangle}{E_i - E_n} + \sum_n \langle i | (E1) | n \rangle \frac{\langle n | H_{PV} | f \rangle}{E_i - E_n} \quad (11)$$

Here $|i\rangle, |f\rangle$ are the initial and final states, E_i, E_f their energies, $|n\rangle$ are a set of intermediate states with opposite parity to $|i\rangle$, and $(E1)$ is the electric dipole operator

$$(E1) = e \sum_j z_j$$

where the sum is over all electrons in the atom.

Two approaches have been used to calculate this sum. In one, proposed by Bouchiat¹ and Bouchiat¹⁰, and applied to Bismuth by Novikov *et al.*¹⁶, the matrix elements of H_{PV} between certain valence electron states are calculated, and the corresponding matrix elements of $(E1)$ are obtained from experiment. This gives a part of the sum involved in equation (11). Corrections to this coming from other excited states are calculated by various approximations. The results agree fairly well with the other method of calculation, described below (Table 1).

In the second approach, used by Henley and Wilets¹⁷ and by Sandars and collaborators¹⁸, the electrostatic interaction between the electrons is replaced by an average central potential, designed to reproduce the observed energy levels of the states. The difference between this central potential and the true electron-electron interaction is treated as a perturbation. This approximation makes it possible to replace the sum over intermediate states in equation (11) by a sum over single particle excitations. Furthermore, by using a technique invented by Sternheimer¹⁹ and others, it is possible to evaluate the sum directly, in terms of the solution to a differential equation.

$$(H_0 - E)|\psi_1\rangle = (E1)|\psi_0\rangle \quad (12)$$

where H_0 contains the approximate central field, and $|\psi_0\rangle$ is an eigenstate of H_0 with energy E . This is a single particle equation. Once it is solved, $M(E1)$ can be expressed as

$$M(E1) = \langle \psi_{1i} | H_{PV} | f \rangle + i \langle \psi_{1f} | H_{PV} | i \rangle \quad (13)$$

These expressions now include a sum over all excited single electron states, discrete and continuum. The results of these calculations are presented in Table 1. They can be seen to agree fairly well with each other, and with the results of the other approach.

However, there are various corrections to these results that must be considered, involving the effect of the residual interaction, which is the difference between the true electron-electron interaction and the approximate central potential. The effects of this residual interaction have been considered in two recent papers^{20,21}. Henley *et al.*²⁰ analysed the effect of the residual interaction on configuration mixing of the valence electron states, and found it to be small, of the order of a few per cent in Bi.

The contribution of the residual interaction to excitation of core electrons has been analysed by Loving and Sandars²¹, and found to be quite large, and opposite in sign to the contribution of equation (11). The discovery of this new contribution leaves the prediction of the standard gauge theory for the Bi optical rotation measurements somewhat in doubt. Loving and Sandars believe they have isolated and correctly estimated the main effects of the residual interaction, giving the overall result quoted in Table 1. If they are correct in this belief, there remains a substantial disagreement between the standard model and experiment. Further calculations as well as experiments using other heavy atoms will be needed to clarify this point.

Experiments in hydrogen and deuterium

Partly because of the difficulty of making accurate calculations for heavy atoms, and partly because the heavy atom experiments are relatively insensitive to C_{2P} , C_{2N} , several experimental groups have attempted to detect a handedness of hydrogen and deuterium atoms. In such atoms, the atomic physics is quite simple, and if an effect is detected, it can easily be analysed theoretically.

The expected amount of handedness in these atoms given by equations (4) and (5) is quite small, and it must be enhanced in order to be detected. One way to do this is to use external magnetic fields to decrease the energy difference between $2S_{1/2}$ and $2P_{1/2}$ in certain hyperfine states^{22,23}. There are several values of the external magnetic field in the neighbourhood of 10^3 gauss at which level crossings occur between these states (Fig. 1). At such level crossings, the real part of the energy denominator in equation (3) vanishes, leaving only the imaginary part, which we have seen is only $1/20$ as great. This means that δ will be 20 times greater than at zero external field. Furthermore, the states which cross at a specific value of the external magnetic field are specific superpositions of hyperfine states. Because of this, the largest value of δ at a level crossing will involve definite linear combinations of C_1 and C_2 , and these combinations will vary from crossing to crossing. This allows the important prospect of measuring both C_{1P} and C_{2P} by measurements in hydrogen, and also measuring C_{1N} and C_{2N} by further measurements in deuterium.

An example of what is involved is provided by an experiment now under way at the University of Michigan²⁴. In this experiment a beam of hydrogen atoms in a specific $2S_{1/2}$ hyperfine state is introduced into a cavity in which are present static magnetic and electric fields, as well as a microwave frequency electric field. The static magnetic field is approximately 550 gauss, at which value there is a level crossing between one of the $2S_{1/2}$ hyperfine states and a $2P_{1/2}$ hyperfine state. The microwave electric field is chosen at a frequency to induce transitions between the initial state and the crossed level. The transition could not, however, occur in the absence of parity mixing because it would be $M1$, and not inducible by the microwave electric field.

It does occur in the actual setup by two distinct paths. The external static electric field can mix $2S_{1/2}$ and $2P_{1/2}$, thus allowing an $E1$ transition from $2S_{1/2}$ to $2P_{1/2}$ by the microwave field. This path involves no parity violation. Alternatively, the $2S_{1/2}$ – $2P_{1/2}$ mixing can occur through H_{PV} , followed by the same $E1$ transition. The transition rate involves an interference between these two paths, and takes the form²⁴, for the specific conditions of the Michigan experiment,

$$R = R_0[1 \pm 2.5 \times 10^{-6} C_{2P}]$$

where R_0 is the rate in the absence of H_{PV} . The \pm sign occurs for different relative orientations of the various electric and magnetic fields. Therefore, if C_{2P} is a number of order 1, as suggested by equation (2), the transition rate will vary by about 1 p.p.m. as the fields are reversed. The experimenters believe that they can detect this small variation in a reasonable amount of running time. Similar experiments are being carried out at Yale University and at the University of Washington. In each case it is expected that the experiments can be done in deuterium as well as hydrogen. The Yale experiment will be sensitive to a different combination of C_1 and C_2 . The successful completion of these experiments will then give the values of each of the four constants that determine H_{PV} .

If further calculations and experiments in heavy atoms and in H and D confirm the Bi results, it will be necessary to modify the theory leading to equations (1) and (2). A number of proposals along this line already exist^{25,26}. Some of these new models

predict that C_{1P} , C_{1N} both vanish, but C_{2P} , C_{2N} do not²⁵. These models lead to greatly decreased effects in heavy atoms but results similar to the standard model in H and D. In other models all four of the C_i are very small or zero²⁶, and therefore predict diminished parity violation effects everywhere. The H and D experiments will obviously be very useful in distinguishing between these models. Some results are expected within the next year.

This research was supported in part by the US Department of Energy.

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articles

Silicalite, a new hydrophobic crystalline silica molecular sieve

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A new polymorph of SiO_2 (silicalite, refractive index 1.39, density 1.76 g cm^{-3}) has a novel topologic type of tetrahedral framework. This encloses a three-dimensional system of intersecting channels defined by 10-rings wide enough to adsorb molecules up to 0.6 nm diameter. Silicalite is hydrophobic and organophilic, and selectively adsorbs organic molecules over water.

A MAJOR scientific and technological achievement since 1949 has been the discovery and development of synthetic crystalline, aluminosilicate zeolites as molecular sieve adsorbents and catalysts. We now report the synthesis, crystal structure, and properties of silicalite, a new microporous crystalline silica with remarkable sieve properties. Unlike aluminosilicate zeolites which are hydrophilic, silicalite is hydrophobic and organophilic, and selectively adsorbs organic molecules in the presence of water.

The crystal structure is a new topologic type of tetrahedral framework, which contains a large fraction of five-membered

rings of silicon–oxygen tetrahedra. The framework outlines a three-dimensional system of intersecting channels defined by 10-rings of oxygen ions in all three directions. Organic quaternary ammonium ions which occupy the channels in the precursor obtained by hydrothermal synthesis, are removed by heating to yield silicalite. The resulting void occupies about 33% of the crystal volume, and the three-dimensional channel is wide enough to adsorb molecules up to about 6 Å in diameter. Silicalite can be heated to near 1,300 °C where it degrades to a glass.

Synthesis

The silicalite precursor is crystallised hydrothermally in a closed system containing alkylammonium cations (for example, tetrapropylammonium), hydroxyl ions, and a reactive form of silica at 100–200 °C. The organic-containing precursor crystals have a typical empirical composition $(\text{TPA})_2\text{O} \cdot 48\text{SiO}_2 \cdot \text{H}_2\text{O}$, mean refractive index 1.48, and measured density 1.99 g cm^{-3} . The organic cation is larger than the pore and therefore must be removed by chemical or thermal decomposition (usually calcination in air at 500–600 °C) to yield the microporous silicalite

crystals (mean refractive index 1.39, density 1.76 g cm^{-3}). As the silica framework is electrically neutral, the organic ion is apparently occluded with hydroxyl ions to maintain charge balance, as indicated by infrared spectroscopy. The unit cell composition for the precursor crystals can be expressed as $[\text{4TPAOH} \cdot 96\text{SiO}_2]$. Unlike aluminium-containing zeolites, silicalite has no cation exchange properties.

The crystallisation mechanism of the precursor seems to involve silica clathration of the hydrophobic organic cation analogous to the formation of crystalline water clathrates of alkylammonium salts^{1,2}. Thus the silica tetrahedra assemble into a framework in place of the hydrogen-bonded water 'lattice' of the water clathrate, and surround the hydrophobic organic guest molecules. The alkylammonium ion seems to enhance the solubility of silica in water in a manner reminiscent of the 'structure-breaking' or 'cluster' forming properties of these same ions in aqueous solution³. Indeed, the alkylammonium ion further links the chemistry and structure of water and silica, and seems to translate the structural chemistry of water below room temperature to silica near 200°C .

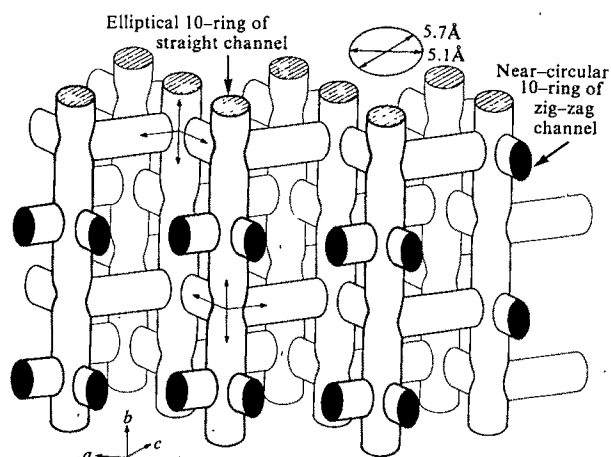
Crystal structure

Precursor crystals are typically $20 \times 20 \times 70 \mu\text{m}$ elongated along c , and commonly occur as interpenetrant twins on the (110) plane. Crystals calcined to 600°C over 2 d have unit-cell edges a , 20.06; b , 19.80; c , 13.36 Å. The systematic absences ($hk0$, $h = 2n+1$; $0k1$, $k+1 = 2n+1$) indicate space group Pnma or $\text{Pn}2_1\text{a}$. Although the symmetry is apparently orthorhombic, weak diffractions may result from either lower symmetry or intergrowth of a second phase. From 8,297 diffraction intensities collected with monochromatised $\text{CuK}\alpha$ radiation out to 2θ 110° with a Picker FACS-1 diffractometer, 3,542 unique diffractions were obtained with 764 above background (3σ). The crystal was twinned, and overlap of diffractions was corrected for the 90:10 ratio of the twin volumes.

Combination of direct methods (MULTAN program) and model building yielded the structure in $\text{Pn}2_1\text{a}$ (Table 1). The 96 tetrahedra per unit cell form a 4-connected framework with a system of intersecting channels (Fig. 1) composed of near-circular zig-zag channels along a (free cross-section 5.4 ± 0.2 Å) cross-linked by elliptical, straight channels along b (free cross-section $5.7\text{--}5.8 \times 5.1\text{--}5.2$ Å). Both channels are defined by 10-rings. The calculated free cross-section assumes that oxygen ions have a radius of 1.3 Å, and depends slightly on the choice of diametrically-opposing oxygens. The channel system same topology as that reported for a 'shape-selective' has the zeolite⁴, but structural details were not given.

Figure 2A is a photograph down the b axis of a model constructed from tetrahedral stars and plastic linkages. Figure

Fig. 1 Idealised channel system in silicalite. To avoid possible confusion caused by the perspective, the dimensions of the channels along c are shown at upper centre.



3A is a topological drawing showing how the framework can be constructed from pairs of tetrahedra, 4-rings and corrugated bands of 6-rings all cross-linked by 5-rings and occasional 6-rings. Figure 3B shows the a -axis projection. The corrugated bands of 6-rings are seen end-on, and each 4-ring lies between

Table 1 Atom positions in silicalite

Atom	x	y	z
SI (1)	0.4225 (6)	0.5468 (3)	0.3518 (9)
SI (2)	0.3055 (9)	0.5150 (10)	0.1944 (7)
SI (3)	0.1902 (2)	0.5570 (2)	0.3197 (6)
SI (4)	0.0687 (7)	0.5225 (8)	0.1616 (9)
SI (5)	0.2160 (8)	0.4223 (8)	0.4595 (10)
SI (6)	0.3752 (8)	0.4401 (10)	0.4627 (10)
SI (7)	0.0788 (8)	0.3597 (10)	0.1744 (10)
SI (8)	0.1888 (10)	0.3056 (10)	0.3060 (10)
SI (9)	0.3166 (10)	0.3582 (10)	0.1586 (10)
SI (10)	0.4266 (9)	0.3193 (10)	0.3227 (10)
SI (11)	0.1220 (10)	0.3138 (11)	0.9710 (10)
SI (12)	0.0754 (7)	0.1208 (8)	0.1912 (10)
SI (13)	0.1965 (9)	0.1517 (8)	0.3032 (10)
SI (14)	0.3187 (10)	0.1150 (10)	0.1766 (12)
SI (15)	0.4228 (10)	0.1631 (10)	0.3005 (12)
SI (16)	0.1184 (9)	0.1586 (10)	0.9420 (11)
SI (17)	0.2727 (8)	0.3166 (9)	0.9773 (11)
SI (18)	0.2752 (7)	0.1658 (9)	0.9599 (11)
SI (19)	0.2267 (9)	0.0492 (10)	0.4464 (11)
SI (20)	0.3845 (9)	0.0592 (8)	0.4818 (11)
SI (21)	0.4249 (8)	0.9393 (10)	0.3132 (12)
SI (22)	0.3063 (9)	0.9575 (10)	0.1701 (11)
SI (23)	0.1881 (9)	0.9450 (10)	0.3239 (12)
SI (24)	0.0590 (8)	0.9051 (8)	0.1989 (10)
O (1)	0.3834 (16)	0.3370 (17)	0.2262 (21)
O (2)	0.3730 (17)	0.5271 (18)	0.2454 (22)
O (3)	0.4837 (18)	0.5483 (17)	0.2972 (23)
O (4)	0.2409 (17)	0.5344 (18)	0.2262 (22)
O (5)	0.3047 (18)	0.4414 (19)	0.1849 (23)
O (6)	0.1082 (19)	0.5474 (18)	0.2573 (22)
O (7)	0.1234 (18)	0.3419 (19)	0.2806 (23)
O (8)	0.2527 (17)	0.3213 (18)	0.2209 (24)
O (9)	0.2995 (18)	0.3313 (17)	0.0822 (23)
O (10)	0.0963 (19)	0.3102 (18)	0.0572 (23)
O (11)	0.1306 (17)	0.1508 (19)	0.2555 (24)
O (12)	0.4967 (16)	0.1527 (17)	0.2721 (22)
O (13)	0.3214 (17)	0.0614 (18)	0.1352 (23)
O (14)	0.3687 (17)	0.1690 (18)	0.2261 (24)
O (15)	0.2481 (18)	0.1473 (16)	0.2212 (23)
O (16)	0.3214 (16)	0.1481 (18)	0.0394 (22)
O (17)	0.0836 (16)	0.4454 (19)	0.1702 (24)
O (18)	0.9931 (18)	0.3393 (18)	0.1904 (25)
O (19)	0.4274 (17)	0.2462 (19)	0.3529 (20)
O (20)	0.3866 (16)	0.4978 (18)	0.4116 (25)
O (21)	0.1827 (17)	0.4747 (18)	0.3853 (22)
O (22)	0.4039 (19)	0.3795 (18)	0.4059 (25)
O (23)	0.1907 (18)	0.3629 (18)	0.4021 (24)
O (24)	0.2892 (16)	0.4112 (17)	0.4760 (20)
O (25)	0.1802 (16)	0.4479 (18)	0.5685 (20)
O (26)	0.4021 (17)	0.4694 (16)	0.5966 (21)
O (27)	0.1974 (18)	0.2503 (18)	0.3459 (20)
O (28)	0.2054 (16)	0.3315 (16)	0.9469 (18)
O (29)	0.0696 (15)	0.3590 (16)	0.9334 (20)
O (30)	0.1011 (12)	0.2550 (16)	0.9533 (20)
O (31)	0.2768 (16)	0.2320 (18)	0.9137 (19)
O (32)	0.0818 (17)	0.1548 (17)	0.0798 (19)
O (33)	0.0766 (18)	0.0362 (18)	0.1714 (22)
O (34)	0.2074 (16)	0.1165 (18)	0.4281 (20)
O (35)	0.3970 (16)	0.1289 (16)	0.3892 (21)
O (36)	0.1917 (17)	0.1492 (17)	0.9844 (20)
O (37)	0.0959 (17)	0.1096 (16)	0.8882 (22)
O (38)	0.2978 (18)	0.3715 (17)	0.8828 (23)
O (39)	0.3030 (19)	0.1275 (16)	0.8621 (20)
O (40)	0.1989 (18)	0.0438 (17)	0.5593 (19)
O (41)	0.4144 (18)	0.0564 (17)	0.5818 (22)
O (42)	0.3853 (17)	0.9245 (18)	0.2291 (19)
O (43)	0.2588 (16)	0.9544 (18)	0.2675 (19)
O (44)	0.1171 (16)	0.9156 (17)	0.2414 (19)
O (45)	0.5020 (16)	0.9461 (17)	0.2986 (22)
O (46)	0.3103 (17)	0.0342 (16)	0.4894 (19)
O (47)	0.4198 (16)	0.9896 (17)	0.3833 (22)
O (48)	0.1992 (18)	0.9823 (17)	0.4196 (21)

Values in parentheses following the values for the atom parameters are the error values. The temperature factor was fixed at 1.0 for the silicon atoms and 2.5 for the oxygen atoms.

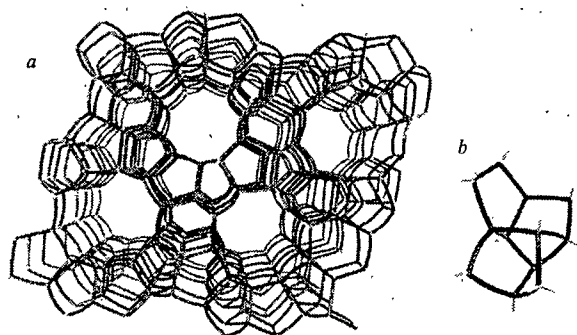
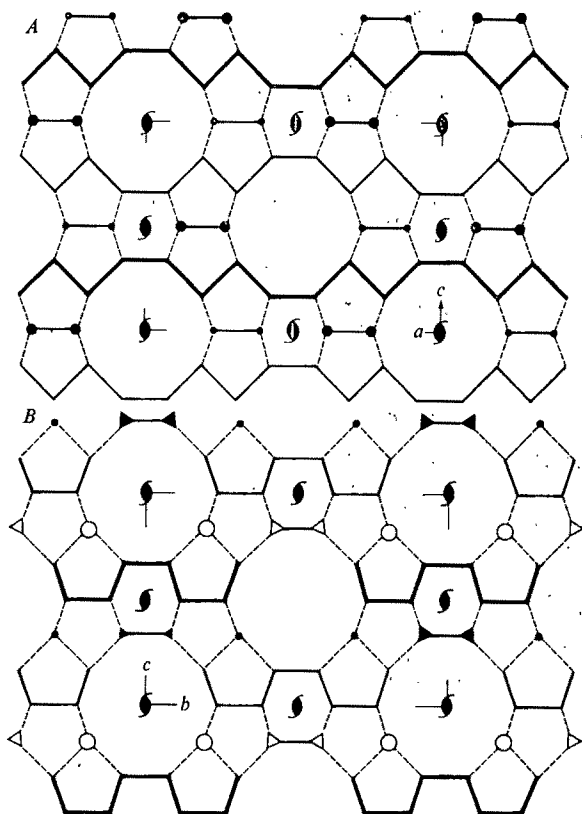


Fig. 2 A, Framework viewed down *b*; B, Secondary building unit of structure. Twelve tetrahedra linked into five 5-rings and one 6-ring.

two linked pairs. Particularly interesting is the high percentage of 5-rings, a feature shared with the zeolites dachiardite, mordenite, ferrierite and epistilbite. Two other polymorphs of silica, coesite and melanophlogite, also contain 5-rings. The frameworks of the zeolites listed above contain an infinite sheet of linked 6-rings, which might be regarded as a subunit involved in synthesis^{5,6}. Perhaps the corrugated bands of 6-rings may be regarded as a sub-unit in the synthesis of silicalite. Alternatively, the silicalite structure can be assembled from subunits of 12 linked tetrahedra (Fig. 2B).

The structural subunits of silicalite can be assembled into other structure types, as will be described elsewhere. These theoretical frameworks give calculated X-ray powder patterns which do not match the observed X-ray powder pattern for

Fig. 3 Topologic drawings of silicalite structure. A, *b*-axis projection, B, *a*-axis projection. Si atoms lie at the intersections of the lines, and O atoms approximately half-way along the lines. Triple bands of corrugated 6-rings are shown by thick and thin continuous lines. Pairs of tetrahedra and 4-rings occur in projection in (B) as dots and circles and as linked open and filled triangles. In (A) the pairs of tetrahedra and the 4-rings superimpose as linked dots of two sizes. The corners of the unit cell are shown, together with screw diad axes of symmetry.



silicalite. The present structure gives a calculated pattern which fits well with the observed pattern (Fig. 4). Because of the weakness of intensities from the small crystal used for the structure determination, the *R* factor is rather high at 0.16. The twinning of silicalite around {110} may be due to faulting perpendicular to *b* allowing a pseudo-tetragonal framework that permits *a* and *b* axes to interchange giving the appearance of a (110) twin axis. An 'hourglass' zoning is also seen on the (010) and (100) faces. The calculated density is 1.80 g cm⁻³ in good agreement with the measured 1.76 g cm⁻³.

Adsorption

Silicalite is a molecular sieve adsorbent with an adsorption pore size near 6 Å and a saturation adsorption pore volume of 0.19 cm³ g⁻¹ in agreement with the properties expected from its crystal structure. At ambient temperature, it adsorbs molecules as large as benzene (kinetic diameter 5.85 Å) but rejects molecules larger than 6 Å, such as neopentane (kinetic diameter 6.2 Å). Although the pore-size effect can be used in molecular sieving, its most remarkable adsorption property is surface selectivity. In contrast to the extremely high preference of aluminosilicate zeolite surfaces for water (hydrophilic) and other polar molecules, silicalite has a very low selectivity for the adsorption of water and a very high preference for the adsorption of organic molecules smaller than its limiting pore size. This hydrophobic and organophilic selectivity manifests itself in several ways. Adsorption of organic molecules and permanent gases on silicalite occurs by the volume filling of micropores as in zeolite molecular sieves and other microporous adsorbents. The filling of micropores occurs by physical adsorption at low relative pressures, and is characterised by enhancement of the adsorption energy due to increase of dispersion forces resulting from comparable size of the adsorption volume and the adsorbed molecule. This results in a type I, near rectilinear isotherm as illustrated for the gas phase adsorption of *n*-hexane on silicalite (Fig. 5b). Pore filling is essentially complete at a relative pressure of 0.03. In contrast, water does not fill the pores at any relative pressure (Fig. 5a). The adsorbed water volume at a relative pressure near one is about 25% of the saturation pore volume for *n*-hexane. In liquid or gaseous mixtures of organic molecules and water, silicalite selectively adsorbs the organic molecule, and thus is capable of removing organic molecules from organic-water streams.

Most solid surfaces are hydrophilic. Previously known hydrophobic surfaces⁷ include graphitised carbon and microporous and macroporous silicas which have been rendered hydrophobic by removal of the hydrophilic surface hydroxyl groups either by thermal dehydration and dehydroxylation or by chemical modification of the surface to replace the hydroxyl

Table 2 Adsorption volumes in silicalite

Adsorbate	Temperature (°C)	Kinetic diameter (Å)	V_p (cm ³ g ⁻¹)	V_t	Molecules adsorbed per unit cell
H ₂ O	RT	2.65	0.047	0.083	15.1
O ₂	-183	3.46	0.185	0.326	38.0
CH ₃ OH	RT	3.8	0.193	0.340	27.6
<i>n</i> -Butane	RT	4.3	0.190	0.334	10.9
<i>n</i> -Hexane	RT	4.3	0.199	0.350	8.8
SF ₆	RT	5.5	0.167*	—	10.9
C ₆ H ₆	RT	5.85	0.134	0.236	8.7
Neo-pentane	RT	6.2	0.029	0.051	1.4

V_p (cm³ g⁻¹) = Total micropore volume in activated silicalite from saturation capacity, calculated using normal liquid densities at the adsorption temperature. Void fraction, $V_t = V_p$ (cm³ g⁻¹) × d_e (g cm⁻³), where d_e is the measured density 1.76 g cm⁻³. All samples activated by calcination in air at 600 °C followed by vacuum activation (10⁻³ torr). Adsorption measurements all by gravimetric McBain-Bakr balance technique. RT, room temperature.

*At 760 torr.

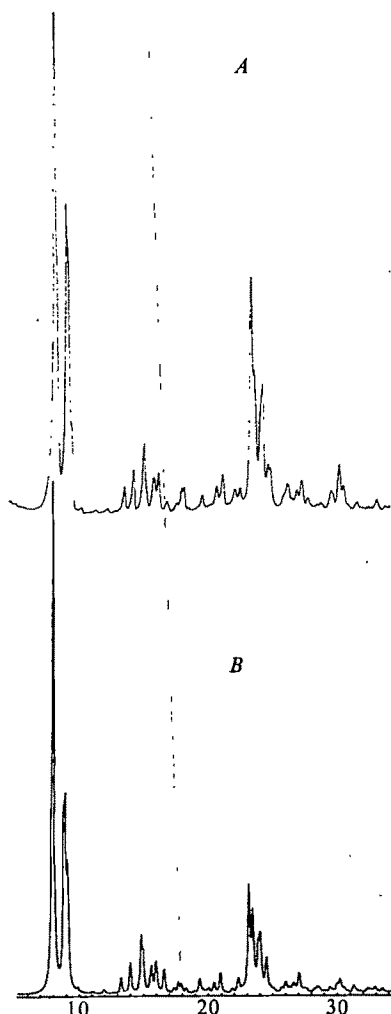


Fig. 4 X-ray powder diffraction pattern of *A*, calcined silicalite material, and *B*, calculated from parameters in Table 1. CuK α radiation with scale in degrees 2θ .

groups with hydrophobic organic or organosiloxane groups. The water isotherms (Fig. 5A) for Graphon, a dehydrated 'HiSil' silica, and a carbon molecular sieve with a pore size (5.0–5.5 Å) and pore volume (0.20 cm³ g⁻¹) comparable to that of silicalite, suggest that the silicalite surface is similar to or more hydrophobic than the Graphon and dehydrated HiSil surface. The carbon molecular sieve (commercial Pittsburgh activated carbon, Calgon Type MSC-V) is more hydrophilic.

The nature of a hydrophobic adsorbent surface has become understood within the last decade and is reviewed comprehensively in ref. 7. For physical adsorption on an ionic surface, the adsorption interaction energy consists of the sum of dispersion and repulsion energies which originate from non-specific interactions, and electrostatic polarisation, dipole and quadrupole energies which represent specific interactions. For adsorption of water, specific interactions are especially important. In the absence of surface sites which are 'hydrophilic', or of sites for hydrogen bonding, polar or acid-base interactions, the surface becomes nonspecific, homogeneous and hydrophobic. In water, each molecule is hydrogen-bonded to its neighbours (approximately four, as in ice), but in silicalite the narrowness of the channels allows interaction with only about two to three molecules on the average. Because silicalite is electrically neutral, there is no strong interaction with water molecules, and energetically the molecules prefer to remain as a liquid outside the silicalite. What small amount is adsorbed in silicalite is probably associated with the residual hydroxyl groups which persist after thermal removal of the

organic ion in the precursor. The initial isosteric heat of adsorption of water on silicalite is about 6 kcal mol⁻¹, substantially below that of the heat of liquefaction of water (9.7 kcal mol⁻¹), and similar to that reported for Graphon⁷. This requires a high entropy of adsorption, again like Graphon. Low energy and high entropy of adsorption (weak adsorption of highly entropic water) indicate high mobility of the adsorbed water molecule. The strongly hydrophilic nature of aluminosilicate zeolite molecular sieves is due to the presence in the intracrystalline void space of polar groups such as cations and hydroxyl groups, and field gradients generated by the substitution of aluminium for silicon in the tetrahedral framework. Silicalite has no aluminium and no cations in its structure. Chen⁸ has shown a substantial decrease in the amount of water adsorbed on the zeolite mordenite (Zeolon) due to the removal of cations, as well as aluminium from the aluminosilicate framework,

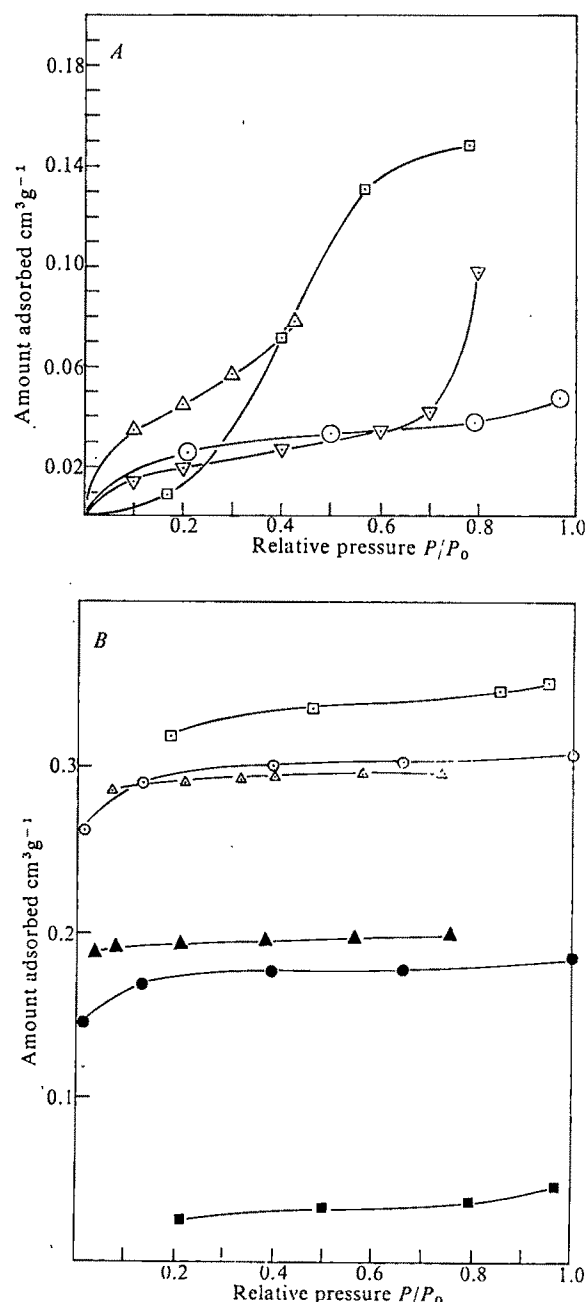


Fig. 5 *A* Water adsorption isotherms on hydrophobic surfaces. □, Carbon molecular sieve; Δ, Graphon; ▽, Hi Sil; ○, silicalite. *B*, Adsorption isotherms on silicalite and NaX zeolite. O₂ at -183 °C, samples activated at 350 °C, 10⁻³ torr; McBain-Bakr gravimetric measurements. □, H₂O on NaX; ○, O₂ on NaX; Δ, *n*-hexane on NaX; ▲, *n*-hexane on silicalite; ●, O₂ on silicalite; ■, H₂O on silicalite.

and described the resulting highly siliceous zeolites as 'hydrophobic'.

Adsorption of *n*-hexane in contrast is highly energetic with isosteric heats of adsorption of 16–18 kcal mol⁻¹ over a wide range of relative pressure. This range is substantially above the heat of liquefaction of *n*-hexane (7.8 kcal mol⁻¹), and similar to the isosteric heat of adsorption of *n*-hexane on the molecular sieve zeolite X, again illustrating the high dispersion energy interactions in crystalline molecular sieves where the adsorption cavities and pores are ≤ 10 Å, commensurate with the size of the adsorbed molecule. Consideration of the volume, size and geometry of the void in the silicalite structure (Figs 1–3) and the number and size of *n*-hexane molecules (8.8 molecules per unit cell and 4.5 Å kinetic diameter) shows that the molecules must be highly oriented in nearly linear strings one molecule thick. The fit of *n*-hexane in the channels is near perfect, and *n*-hexane becomes a low entropy highly ordered liquid in the silicalite lattice. Typical adsorption volumes for a variety of molecules on silicalite are given in Table 2.

Stability

Silicalite possesses a remarkable stability for a 33% porous crystal. It is stable in air to over 1,100 °C, and only slowly converts to an amorphous glass at 1,300 °C. It is stable to most mineral acids but reacts with HF similarly to quartz. X-ray emission measurements of the SiK β band show that the mean Si–O bond energy of silicalite exceeds that of quartz by 0.1 eV,

and is essentially the same as for cristobalite. In contrast, the mean Si–O bond energy in aluminosilicate zeolites is substantially less than for quartz⁹.

Applications

Thus silicalite may offer practical applications in the clean-up of water contaminated with organic compounds. Traces of methanol, propanol, butanol, phenol, 1,4-dioxane, pentane and hexane have been removed from water. The selectivity of silicalite is nearest to that of adsorbent carbons, but it has the advantage of much higher stability to regenerative commercial processes involving thermal, acid, and oxidative conditions.

We thank T. R. Cannan for his assistance in synthesis and adsorption characterisation, and Union Carbide Corporation for permission to publish this article.

Received 18 November; accepted 30 December 1977.

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Radon emanation on San Andreas Fault

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Subsurface radon emanation monitored in shallow dry holes along an active segment of the San Andreas fault in central California shows spatially coherent large temporal variations that seem to be correlated with local seismicity.

RADON is constantly emanated from the Earth into its atmosphere, normally in minute amounts. Radon emanation is known to be anomalously large on active faults and to show temporal variations related to changing atmospheric conditions and possibly nearby seismic activities^{1,2}. To check whether it may show premonitory changes useful for earthquake prediction like those reported for the emanation from deep aquifers^{3,4}, the US Geological Survey began on 7 May 1975 to monitor subsurface radon emanation in 20 shallow dry holes along an active 60-km segment of the San Andreas and Calaveras faults in central California (stations 1–20, Fig. 1). Here I present some initial results of this experiment and discuss possible explanations.

We used a simple track etch method^{5–7} that had been developed for uranium exploration to measure radon emanation: a small piece of plastic film (cellulose nitrate) which is sensitive to α radiation is attached to the inside bottom of a plastic cup (9 cm high, 7 cm aperture). The cup is placed upside down at the bottom of a borehole (10 cm in diameter, 0.7 m deep) to expose the film to the soil gas for 1 week, after which it is replaced by a new cup. The air gap in the cup is sufficient to shield the film from all α particles generated in the soil. As radon and its isotopes are gaseous, however, they may move into the cup to emit α particles close enough to leave tracks in the film. The retrieved film is then chemically etched and the enlarged α -particle tracks in the film within an area of nearly 6 mm² are counted under a microscope. The measured track density is then assumed to be proportional to the average radon

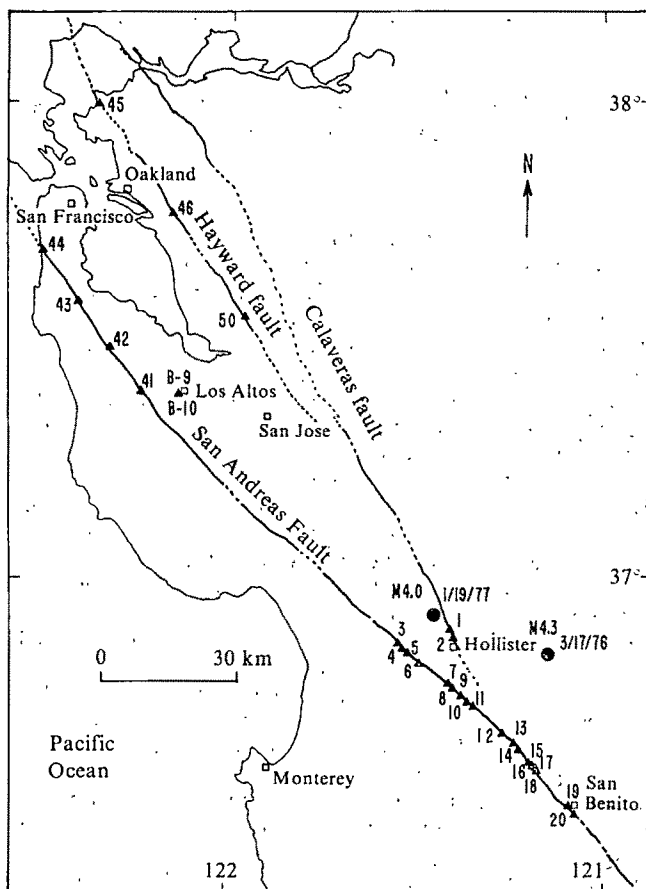


Fig. 1 Location of radon monitoring stations in central California (▲ with station identification numbers) and epicentres of two larger earthquakes (● with magnitudes and dates).

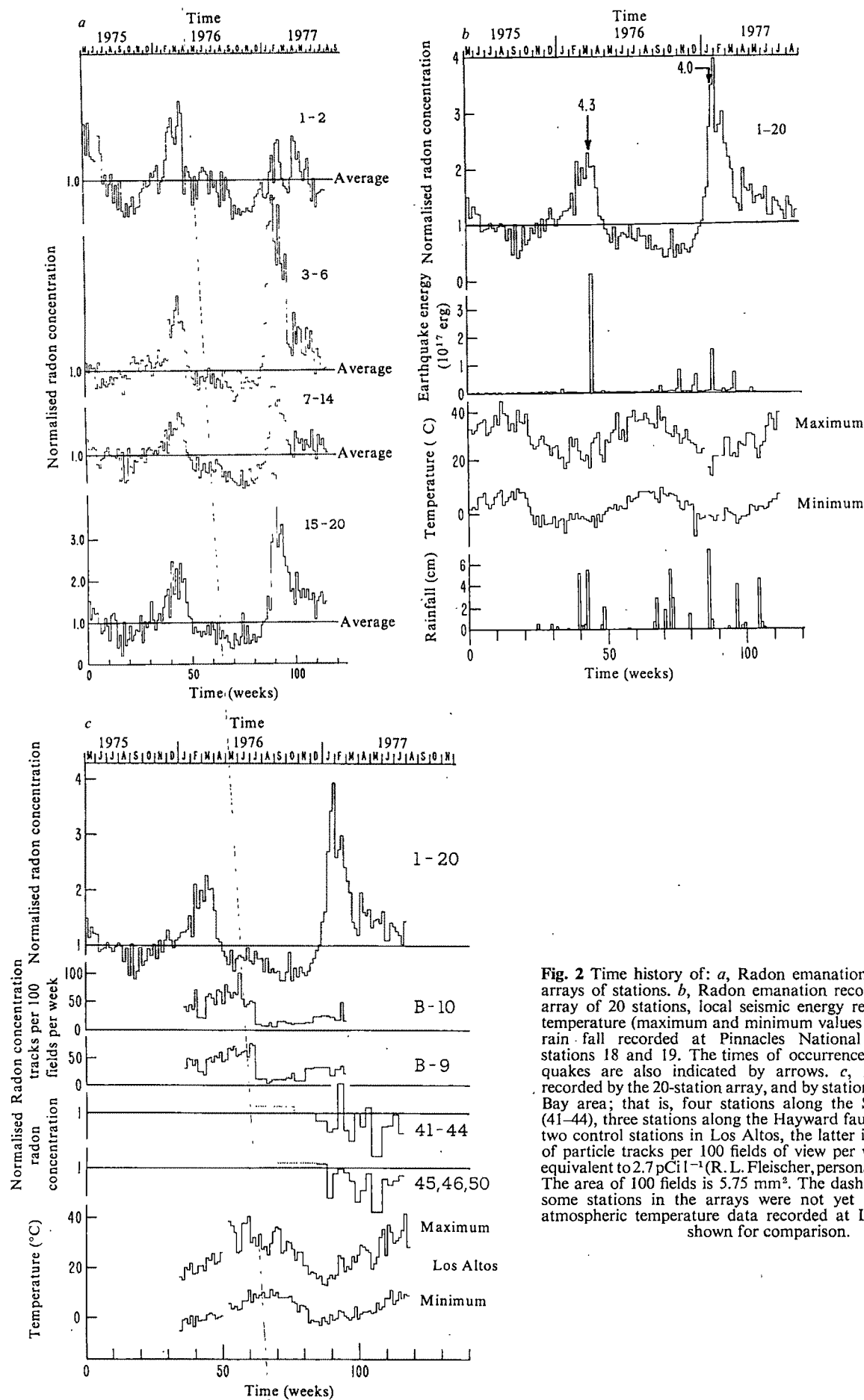


Fig. 2 Time history of: *a*, Radon emanation recorded by four arrays of stations. *b*, Radon emanation recorded by the whole array of 20 stations, local seismic energy release, atmospheric temperature (maximum and minimum values of each week) and rain fall recorded at Pinnacles National Monument near stations 18 and 19. The times of occurrence of the two larger quakes are also indicated by arrows. *c*, Radon emanation recorded by the 20-station array, and by stations in San Francisco Bay area; that is, four stations along the San Andreas fault (41-44), three stations along the Hayward fault (45, 46, 50), and two control stations in Los Altos, the latter in units of number of particle tracks per 100 fields of view per week. Each unit is equivalent to 2.7 pCi l^{-1} (R. L. Fleischer, personal communication). The area of 100 fields is 5.75 mm^2 . The dash lines indicate that some stations in the arrays were not yet in operation. The atmospheric temperature data recorded at Los Altos are also shown for comparison.

concentration of the soil gas in the hole during the period of measurement. In the following, I shall not differentiate between the various contributions to the recorded α radiation by radon and by the isotopes thoron and actinon which have much shorter half lives (55 s and 4 s, respectively) than radon (3.8 d).

The boreholes are supported by plastic pipes 0.8 m long. The upper ends of the pipes are approximately 0.1 m above ground surface and are capped to prevent surface water from flowing into the holes and to reduce possible atmospheric effects. A small amount of moisture was sometimes observed on the retrieved films, especially during the springs for these stations, but it does not seem that the moisture has caused any serious detection problems such as reduced sensitivity, since higher radon emanation was recorded during the springs at these stations. The monitoring sites are located mostly in the fault zones for the purpose of maximising possible tectonic signals, because tectonic strain changes are likely to be amplified in a fault zone due to the mechanical weakness of the zone⁸.

The measured weekly average values of radon concentration of soil gas ranged from 5 to 2,300 pCi l⁻¹. Figure 2a shows radon concentration as a function of time for the 20 stations grouped into four small arrays (stations 1–2 on the Calaveras fault, stations 3–6, 7–14 and 16–20 from north-west to south-east along the San Andreas fault) to improve the signal-to-noise ratio. Data of each array are obtained by first dividing the weekly readings of each station by the average value of the station during the first 40 weeks, and then by summing these 'normalised' values of the same week for all the stations in the array with equal weight.

It is evident from Fig. 2a that the measured radon emanation shows large temporal variations that are spatially coherent over long fault segments (≥ 60 km). The radon variations are not correlated with the relatively small amount of rainfall (Fig. 2b). Also they do not seem to correlate significantly with barometric pressure changes, presumably because of the integrated nature of the measurement, each value representing a weekly average. But because the two prominent peaks in the radon data are separated by nearly a year (46 weeks), one may suspect a seasonal effect, possibly caused by atmospheric heating and cooling of the ground. As shown in Fig. 2b, the correlation between the recorded radon emanation and the atmospheric temperature seems to be exceptionally close. Other observations suggest, however, that this correlation may well be fortuitous. If, for example, the observed temporal radon variations are due mainly to a seasonal effect, one might expect to see fairly similar variations in climatically similar areas, though differences can be caused by different geological conditions at individual sites. Figure 2c shows the radon data recorded by stations that were later installed in the San Francisco Bay area (Fig. 1). These include four stations along a 'locked' segment of the San Andreas fault (41–44), three stations along the Hayward fault (45, 46, 50) and two control stations that are only 3 m apart in Los Altos which is about 8 km off the San Andreas fault. The temperature data recorded at Los Altos are also shown in Fig. 2c for comparison. The radon emanation there apparently varied quite differently, although the temperature variations are similar to those recorded in the 20-station area.

Figure 2b also shows the weekly energy release by earthquakes of magnitude 1.0 and larger within 30 km of the network computed from an energy-magnitude relation given by Richter⁹. The correlation between the radon and the seismicity data seems to be reasonably good. The two largest quakes (magnitude 4.3 and 4.0, respectively), as indicated by arrows in Fig. 2b and solid circles in Fig. 1) occurred near the times when the radon emanation reached its peaks. The emanation began to increase rapidly several weeks before the magnitude 4.3 quake when the smaller quakes did not yet increase in number. This result may indicate that the increase in radon emanation is not due to seismic shaking but may possibly be due to strain build up. In contrast, both radon emanation and seismicity increased more or less concordantly during the second 'anomalous'

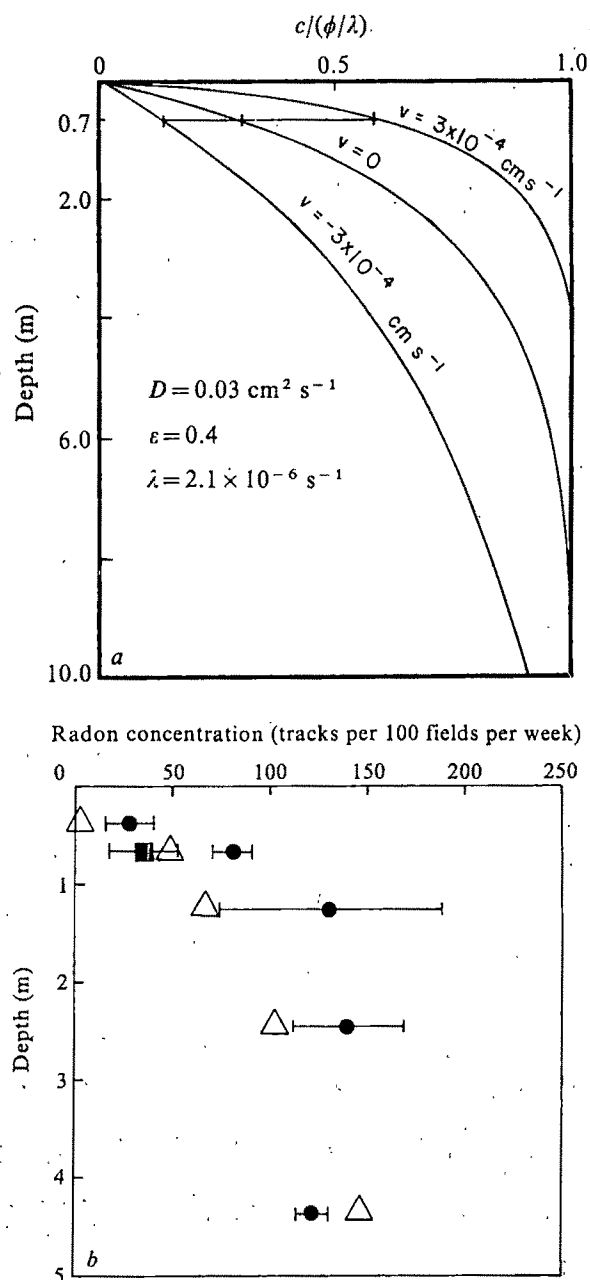


Fig. 3 Radon concentration of subsurface soil gas as a function of depth, *a* for a theoretical model (after Clements¹¹); *b*, observed at site 20. The square indicates a long-term average value at the usual monitoring depth of 0.7 m. ●, Indicate the result for an anomalous period (weeks 96–98) and the triangles the result for a nearly normal period (weeks 99–100). The bars indicate 1 σ .

period in which the magnitude 4.0 quake occurred.

From the available data, I suggest that the observed radon variations are more likely to be related to those of seismicity than atmospheric temperature.

If the observed radon variations are indeed mainly earthquake related, what is the responsible mechanism? In view of the fact that radon has a short half life (3.8 d) and moves slowly in the ground¹⁰, it is unlikely that the detected radon comes from earthquake sources several kilometres deep. The anomalously high radon emanation, however, may conceivably be due to an increase in crustal compression that squeezes out the soil gas in the deep reaching fault gouge zone into the atmosphere at an increased rate, as an increased outgassing rate may perturb the vertical subsurface radon concentration profile (radon concentration is known to increase rapidly with depth by about 3 orders of magnitude), such that deeper soil gas containing more radon is brought up to the detection level. (The proposed

mechanism for earthquake related radon emanation variations will be discussed in more detail elsewhere.)

To obtain some quantitative estimate of the effect of a vertical soil gas flow on radon emanation, let us consider the one dimensional model by Clements¹¹. In this model the soil layer is considered to be a homogeneous porous half space (that is the lower boundary is much deeper than 10 m) with uniform radon production. Assume radon migrates vertically by a combination of the following two processes: (1) molecular diffusion governed by Fick's law and (2) gas flow governed by Darcy's law. Having taken into account the equation of continuity for radon and the boundary condition that radon concentration is 0 (negligibly small) at the ground surface, Clements obtained the following steady state result for radon concentration C as a function of depth ($-z$):

$$C = \frac{\phi}{\lambda} \left\{ 1 - \exp \left[\gamma \left(\frac{\varepsilon \lambda}{D} \right) z \right] \right\}$$

where

$$\gamma = \frac{v}{2(\varepsilon \lambda D)^{1/2}} + \left(\frac{v^2}{4\varepsilon \lambda D} + 1 \right)^{1/2},$$

ϕ is radon production rate, λ is its decay constant, ε is soil porosity, D is molecular diffusion coefficient of radon in the soil, and v is apparent soil gas flow velocity (volume of flow per unit time per unit geometric area). This result is shown in Fig. 3a for three different flow velocities and some material constants appropriate for typical dry soils. It is evident that a small vertical flow of soil gas (3×10^{-4} cm s⁻¹) can perturb significantly the subsurface radon concentration profile at shallow depths (< 10 m) such that the concentration at the depth of 0.7 m (monitoring depth of this study) is changed by a factor of 2.

To check whether subsurface radon concentration profile varies as predicted, we made radon measurements in boreholes

of several different depths separated by several metres at station 20. Figure 3b, shows the profiles for a 3-week period (week 96–98) during which the radon emanation was still anomalously high (see Fig. 2a), and for a 2-week period (week 99, 100) during which the emanation was nearly back to the average level. It is evident that during the anomalous period radon concentration increased at the shallower depths only, in agreement with the model.

If the observed temporal emanation variations are indeed earthquake related, why are they so large and so spatially coherent for earthquakes that are relatively small? One possibility is that the fault gouge zone, in which most of the radon stations are located, is mechanically much more compliant than the rocks on either side. As a result, strain changes may tend to concentrate in the fault zone over a long segment⁸. Similar spatial coherence is evident in some other fault-zone geophysical data, such as coseismic steps recorded on creepmeters at the times of local earthquakes of comparable magnitudes (> 4.0) (ref. 8) and fault zone deformation episodes indicated by data from alignment arrays¹².

This model may also explain the observed increase in radon emanation resulting from underground nuclear explosions¹³.

I thank W. E. Clements, J. Evernden, R. L. Fleischer, J. H. Healy, C. B. Raleigh and J. C. Savage for helpful comments and W. A. Gaman, C. Walkingstick and B. Williamson for technical assistance.

Received 19 September; accepted 15 November 1977.

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Structural comparison of glycophorins and immunochemical analysis of genetic variants

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Differences in amino acid sequence of erythrocyte membrane glycophorin A are correlated with M or N blood group activity. A second sialoglycoprotein, glycophorin B, has an amino acid sequence identical to that of glycophorin A^N in the first 23 positions and carries N activity only, suggesting that different structural genes code for the glycoproteins carrying these antigens. Certain genetically variant cells lack glycophorin A, as determined by immunochemical methods, and serological MN activity. Other variants lack MN activity, but contain normal amounts of glycophorin A in the membrane.

LANDSTEINER and Levine¹ described a new blood group system in 1927, called MN, in which two alleles *M* and *N* determine the presence of corresponding antigens on the red cells. According to this two-allele theory, three genotypes are possible: *MM*, *MN* and *NN*. In 1947 an additional two-allele antigenic system *Ss* was found²; this is closely linked with *MN* and only rarely have recombination events been observed between *MN* and *Ss*³. The

antigens of the *MN* and *Ss* systems are probably found on different cell surface glycoproteins⁴. There are, however, other data which are inconsistent with this simple genetic interpretation of the serological data. *MM* homozygotes have been shown to express some *N* antigenic activity on the surface of their red cells; this becomes more readily detectable after proteolytic treatment of intact cells⁵. As neuraminidases destroy *MN* activity of human cells, it has been concluded that carbohydrates and sialic acid in particular are essential in the determinants⁶ and that the *MN* alleles code for glycosyltransferases^{7,8}. It has, however, also been shown that various reagents reacting with amino groups destroy *MN* activity and this has led to the idea that protein structures are part of the determinants^{3,4}.

Genetically variant cells have been described serologically, lacking one or the other antigen, and the question arose as to whether absence of the antigen(s) is caused by changes in carbohydrate structure or absence of the glycoproteins carrying the antigenic determinants. We report here studies on the primary structure of glycophorin A isolated from *M* and *N* homozygotes, and the amino terminal segment of glycophorin B. *MN* heterozygotes contain two different glycopeptides in their membrane, which possess different amino acids in positions 1 and 5. The

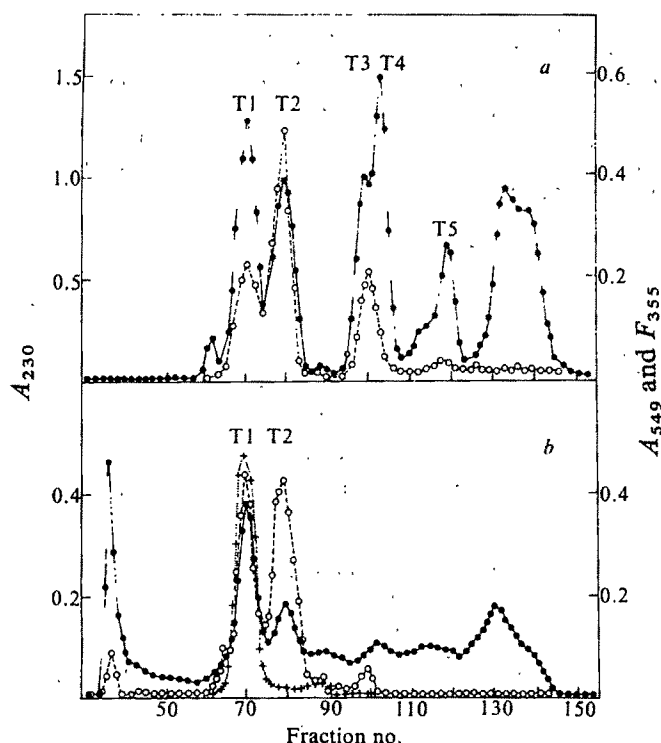


Fig. 1 Separation of tryptic peptides of glycoprotein A (a) and glycoprotein B (b) by gel filtration on Sephadex G150 superfine. 300 mg of glycoprotein A or glycoprotein B (fraction B in Fig. 1, ref. 21) isolated from pooled blood was dissolved in 30 ml of 50 mM Tris-HCl, pH 8.5 and was digested for 24 h with trypsin (TPCKT, Worthington) at an enzyme:substrate ratio of 1:30. After digestion, 3 mg of tosyl-L-Lys-CH₂Cl was added and the pH was adjusted to 4.5 with 1N HCl. The precipitate which formed during acidification was removed by centrifugation and the supernate, containing the soluble peptides, was freeze-dried and after resolubilisation was applied to a column of Sephadex G150 superfine (Pharmacia, Sweden), equilibrated in 0.1 M ammonium acetate at pH 6.8. The column dimensions were 2.4 cm × 150 cm, flow rate 10 ml h⁻¹, fraction volume approximately 5 ml. The column effluents were monitored for protein by measuring the absorbance at 230 nm (●), for sialic acid content by the procedure of Warren¹⁴ at 549 nm (○) and for tryptophan by measuring fluorescence emission at 355 nm (excitation 295 nm), (+).

glycopeptide isolated from glycoprotein B shares the amino acid sequence of glycoprotein A^N regardless of the MN type of the cell of origin. We further confirm previous suggestions that certain genetic variants at the MN locus entirely lack glycoprotein A⁹⁻¹¹, but genetically determined loss of MN activity is not necessarily correlated with the absence of glycoprotein A.

Glycoprotein A^M and glycoprotein A^N have different amino terminal sequences

In previous studies on the primary structure of glycoprotein A, two positions in the sequence consistently gave two amino acids: leucine and serine as the N-terminus and glycine and glutamic acid in position 5. In view of recent controversial findings⁴¹ and suggestions that glycoprotein A from M or N homozygous individuals contains predominantly one N-terminal residue, namely serine or leucine^{18,19}, we have reinvestigated the N-terminal structure of glycoprotein A isolated from two to five individuals, each with genotypes MM, MN, or NN.

Glycoprotein A was isolated by the LIS-phenol method²⁰ and gel filtration in detergent²¹. After tryptic digestion, the insoluble peptide portion was removed^{13,14} and the soluble peptides were separated by gel filtration (Fig. 1a)¹⁴. As described previously, two glycopeptides (T1 and T2) derived from the N-terminal end of the polypeptide chain, were found^{12,14,15}. Peptide T2 is obtained by cleavage at a lysyl and/or arginyl bond of part of the glycoprotein A molecules within the T1 sequence, which is insensitive to trypsin in T1. T2 is thus shortened at the C-terminal end by nine amino acid residues. T1 and T2 were re-

chromatographed by ion-exchange chromatography¹⁴. The yield of combined peptides T1 and T2 for each preparation was approximately 300 nmoles per unit of blood. The amino acid and carbohydrate composition of some of the peptides are given in Table 1. According to these data, T1 (not shown) and T2 from N homozygotes lack glycine, but contain one additional glutamic acid and leucine residue. The same peptides isolated from glycoprotein A from MN heterozygotes seem to be a mixture of both forms and the amino acid composition is comparable to that found in earlier studies^{12,14}. The carbohydrate composition of T1 (not shown), or T2 is rather similar for the three different preparations (Table 1).

The partial amino acid sequence was determined for the first 7 amino acid residues of T2 by simultaneously using the direct Edman degradation and dansyl-Edman procedure^{12,15}. The amino terminal sequence of glycoprotein A^M is Ser-Ser-Thr-Thr-Gly- as compared to glycoprotein A^N which has the sequence Leu-Ser-Thr-Thr-Glu-. The presence of both serine and leucine in position 1 and glycine and glutamic acid in position 5 for T2 isolated from glycoprotein A^{MN} indicates that this preparation is a mixture of the two types.

PTH-derivatives were not found in any preparation in steps 2, 3 and 4, and as described earlier, the finding of dansylated serine and threonine derivatives in these positions indicates that these residues are glycosylated^{12,15}. Figure 2 also contains the rest of the amino acid sequence of T2, which should be identical for all three preparations, since the amino acid composition does not indicate further heterogeneity and none was observed in this region of the sequence in our earlier studies^{12,15}. Quantitation of PTH-derivatives by gas chromatography was possible only for the non-glycosylated amino acids and we found for A^M-T2 90 nmol of serine in position 1, 13 nmol of glycine in position 5 and 64 nmol of valine in position 6. The uncorrected values of the amino acids found in these positions for A^N-T2 are 75, 39, and 23. No other amino acids were found, indicating that the isolated peptides are homogeneous with respect to the protein structure.

Table 1 Amino acid and carbohydrate analysis of N-terminal peptides of glycoprotein A and glycoprotein B*

	A ^M -T2	A ^N -T1	B ^{MN} -T2
Asp	2 (2)	2 (2)	1.3(1)
Thr†	7 (7)	7.1(7)	7.2(7)
Ser	8.7(9)	7.9(8)	8.3(8)
Glu	1.2(1)	2.3(2)	4 (4)
Pro	—‡	—	0.2(—)
Gly	1 (1)	—	2.2(2)
Ala	1.1(1)	1.1(1)	1.2(1)
Cys	—(—)	—	—(—)
Val	2 (2)	2.1(2)	2.7(3)
Met§	0.4(1)	0.4(1)	0.6(1)
Ile	0.9(1)	1 (1)	0.9(1)
Leu	—(—)	1.1(1)	2 (2)
Tyr	0.7(1)	0.8(1)	0.6(1)
Phe	—(—)	—(—)	—(—)
His	2 (2)	2 (2)	1.7(2)
Lys	2 (2)	1.9(2)	1.2(1)
Arg	0.5(—/1)	0.6(—/1)	0.7(1)
Total	30	30	35
Carbohydrate composition			
Fuc	0.6	0.2	—
Man.	2.8	2.6	—
Gal	11.9	10.5	10.7
GalNac	8.3	7.6	13.8
GlcNac	5.4	5.1	—
Nana	9.8	10	15.8

*Amino acid analysis was done on a Durrum D500 analyser, using norleucine as an internal standard. The values are given in mol per mol peptide with expected values in brackets.

†Values for Thr and Ser are corrected for losses during hydrolysis by extrapolation to zero time after 24, 36, and 48 h hydrolysis.

‡Dash denotes 0.1 or less.

§Uncorrected values for methionine.

||Mol per mol peptide. Carbohydrates were determined as trimethylsilyl derivatives of methyl glycosides by gas-liquid chromatography, on a Hewlett-Packard 5110 B gas chromatograph, using inositol as an internal standard¹².

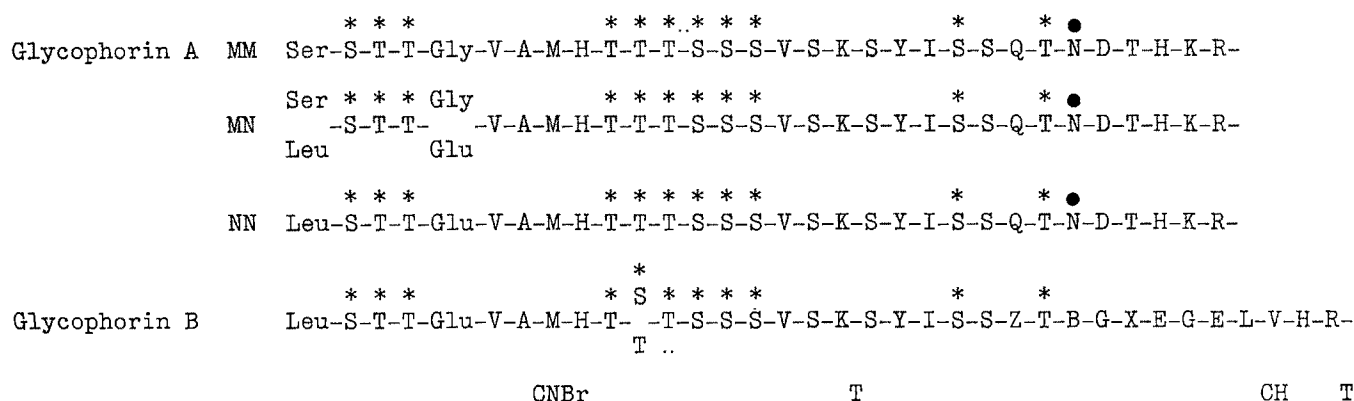


Fig. 2 Amino acid sequence and sites of glycosylation (* ●) of the N-terminal region of glycophorin A and glycophorin B. ↑ cleavage sites: cyanogen bromide (CN Br), trypsin (T) or chymotrypsin (CH). ←, residues identified after dansyl-Edman degradation by thin layer chromatography. →, residues identified as PTH-derivatives by gas-liquid chromatography. Except from positions 1 and 5, the one later code for amino acids is used⁴³.

N-terminal sequences of glycophorin A^N and glycophorin B are identical

Glycophorin B is not yet as well defined as glycophorin A. This sialoglycoprotein can be distinguished by its different electrophoretic mobility on SDS-polyacrylamide gels^{23,24}, amino acid composition²¹ and antigenic properties (refs 16, 17, and unpublished observations) and can be separated from glycophorin A by gel filtration in detergents²¹ or elution from SDS-polyacrylamide gels¹⁰. In certain genetically variant cells which lack both serological Ss activity and the trace N activity normally associated with *MM* homozygotes^{5,16}, periodic acid Schiff (PAS) staining bands in the glycophorin B positions are absent²⁹.

It has not yet been possible to isolate electrophoretically pure glycophorin B in preparative amounts. We decided therefore to use fractions B and C (Fig. 1 in ref. 21) obtained by gel filtration of the entire sialoglycoprotein fraction, as starting material for the isolation of glycopeptides. These fractions are highly enriched for glycophorin B, but contain variable amounts of a third and possibly other minor sialoglycoproteins, as suggested previously (refs 22–24, and unpublished observations). Figure 1b shows the chromatogram of the soluble peptides of a tryptic digest of fraction B. The two glycopeptides which elute in this system at positions identical to T1 and T2 of glycophorin A, were further purified by ion-exchange chromatography on DEAE-cellulose in 50 mM sodium formate, pH 6.1, and were eluted with a linear salt gradient (0–0.3 M NaCl). Under these conditions, peptides T1 (or T2) from glycophorin A are separated and thus contamination of the glycophorin B preparation with glycophorin A can easily be monitored.

Peptide B-T2 was also isolated as the major peptide component from a tryptic digest of fraction C (see Fig. 1 in ref. 21), which contains almost pure glycophorin B (data not shown). The amino acid and carbohydrate composition of glycopeptide B-T2 is given in Table 1, which shows that B-T2 is closely related to A-T2 in amino acid composition, but lacks mannose and N-acetyl glucosamine entirely. In contrast, B-T1 contains tryptophan, which is not found in glycophorin A, has an amino acid composition quite different from that of A-T1, but contains mannose and N-acetyl glucosamine (not shown). Further work will be required to establish whether it is derived from glycophorin B or yet another sialoglycoprotein.

We have determined the amino acid sequence of B-T2 by the methods described above. In order to accomplish this, B-T2 was desialated with neuraminidase and was cleaved with cyanogen bromide, trypsin and chymotrypsin^{12,14}, and the peptides indicated by arrows in Fig. 2 were obtained. A more detailed description of these data will be published elsewhere. The secondary peptides as well as B-T2 (steps 1–16) were subjected to manual Edman degradation and we found an amino acid sequence which was identical in at least the first 23–25 amino terminal residues to

glycophorin A^N, despite the fact that this peptide was obtained from glycophorin B isolated from pooled blood of many individuals. Also, the threonine and serine residues glycosylated in glycophorin A were found to be glycosylated in exactly the same positions within the first 25 amino acid residues. B-T2 contains one asparagine or aspartic acid. The lack of glycosylation at this position could be explained either by finding only aspartic acid in this position or by the drastic change in amino acid sequence C-terminal of residue 26, which is entirely different from the corresponding region of glycophorin A. N-glycosylation as far as known invariably occurs at asparaginyl residues in the sequence Asn-X-Thr (or Ser) (ref. 25). On occasion we found serine in addition to threonine in position 12. Since the residue in this position is glycosylated, only dansylation detects an amino acid. The lack of quantitation does not allow us to decide whether serine is the only amino acid residue at this position.

Serological MN activity correlated with amino terminal structure of glycoproteins

As indicated earlier, many laboratories have reported the presence of N-activity on *MM* homozygous cells^{5,7,10}, and this finding is one of the crucial elements for a hypothesis that the N-blood group substance represents the precursor for M⁷. We therefore studied the blood group activities which are associated with these proteins, isolated from individuals which are classified according to the two allele model into *MM*, *MN* and *NN*. First attempts focused on the activities of glycophorin A and glycophorin B preparations isolated as described above. A^N and B^N did not inhibit the agglutination of *MM* erythrocytes, but B^M inhibited the agglutination of *NN* erythrocytes, suggesting that *MM* homozygotes contain N activity in one of the minor sialoglycoproteins as previously suggested⁴. However, the difference between the activity of B^M in inhibiting *MM*- and *NN*-agglutination was only 3–4-fold, suggesting that B^M is contaminated with A^M, for which our chemical studies provided sufficient support.

In view of the difficulties in isolating pure glycophorin B, we analysed the serological activities of the tryptic glycopeptides which can be purified and characterised more readily. In Table 2 the data obtained by a semiquantitative haemagglutination-inhibition assay are summarised and the results can be stated as follows: the total glycophorin fraction, glycophorin A and the N-terminal peptides isolated from glycophorin A inhibit the homologous agglutinating system, but not the heterologous system; that is, peptides isolated from *M* homozygotes inhibit only the agglutination of *MM* cells, but not of *NN* cells. Peptides prepared from glycophorin A^{MN} are equally active in both systems; only the amino terminal glycopeptides are active, but not the only other glycopeptide T3A derived from glycophorin A, which is also located at the exterior of the cell^{12,14}; peptides T1 and T2 are equally active, but are 2–4-fold less active than glycophorin A,

Table 2 Serological activity of sialoglycopeptides isolated from glycophorin A and glycophorin B in individuals homozygous or heterozygous at the *MN* locus

Inhibitors	Haemagglutinin-inhibition of inhibitors*	
	MM cells anti- <i>M</i> serum†	NN cells anti- <i>N</i> serum†
MM Glycoprotein fraction‡§	1.5	> 36
A ^M §	2.1	> 25
A ^M -T1	9.4	> 226
A ^M -T2	10.8	520
A ^M -T3A¶	> 2,250	> 2,250
A ^{MN} -T1	n.t.	n.t.
A ^{MN} -T2	20.6	13.7
NN Glycoprotein fraction§	> 40	1.2
A ^N §	> 30	3.7
A ^N -T1	> 90	7.5
A ^N -T2	> 160	6.7
B ^M	6.2	6.2
B ^{MN} -T1¶	> 384	> 384
B ^{MN} -T2¶	> 440	6.9

Sialoglycoproteins and glycopeptides were assayed by a semi-quantitative haemagglutination-inhibition assay. One drop each of serum appropriately diluted (four agglutinating units) was incubated with one drop of a serial dilution of inhibitor for 3 min, before one drop of a 5% erythrocyte suspension in phosphate buffered saline was added. The plates were shaken at room temperature and were scored after 10 min. The protein concentration of the inhibitors was determined by amino acid analysis using norleucine as internal standard.

*Concentration of inhibitors in nmol ml⁻¹ completely inhibiting four agglutinating units. n.t. not tested.

†Sera obtained from Ortho, Lot No. M 127, M 173, and N 157.

‡MM, MN, and NN refer to the serological blood type of the red cell membranes, which were used to isolate the total sialoglycoprotein fraction, glycophorin A (A^M or A^N) or glycophorin B (B) by methods described elsewhere^{20,21}. Tryptic sialoglycopeptides T1, T2, T3A were prepared from glycophorin A or glycophorin B (A-T1 etc., B-T2 etc.)

§Single donors.

||Pooled blood from two donors.

¶Pooled blood from about 25 donors.

which is about 1.5- to 3-fold less active than the unseparated mixture of sialoglycoproteins; only the amino terminal peptide B-T2 of glycophorin B shows activity and this activity is restricted to *N*. The starting material for the isolation of peptide B-T2, glycophorin B, has activity for *M* and *N*, consistent with the phenotype of the original cells from which it has been isolated and assuming that glycophorin A is present in this fraction as a contaminant.

Genetic variants which lack glycophorin A

In Table 3 sialoglycoprotein profiles of membranes from some variant cells are calculated from SDS-polyacrylamide gels²⁶ in comparison with normal cells. The cells we have used are either homozygous or heterozygous at the major *MN*-locus and according to serological data express either no *MN* activity or only half the amount³. On the basis of this analysis, several cell types can be distinguished: heterozygous *M^NN* cells which serologically express only *N*, but no *M* activity, are indistinguishable from normal cells; heterozygous *M^kM* cells³, which serologically express only half the activity of *MN* and *Ss* antigens, have only 50% PAS-stainable protein as compared to normal cells, affecting both glycophorin A and B; cells from two individuals designated *En(a-)* G. W. (Finland) and *En(a-)* E. H. (England) are indistinguishable from each other and show a reduction of the total stainable glycoprotein of about 80%, mostly accounted for by loss of glycophorin A stainability; cells with *Tn* phenotype^{3,28,42} again show about 80% reduction of stainable glycoproteins, which affects both glycophorin A and B. Although these results are in general agreement with serological results (with the exception of *En(a-)* E. H. (refs 30, 40)) and with the finding of others, several problems inherent in this technique should be pointed out.

Tanner and Anstee⁹ first suggested that the major sialoglycoprotein is absent in *En(a-)* cells. Dahr and colleagues in a series of papers concluded from their analysis of gel electrophoretic data (similar to ours) in conjunction with serological results that the serological abnormalities observed in these and other variant cells are correlated with the partial or total absence of one or more of the cell membrane sialoglycoproteins^{10,29,30}. Gahmberg *et al.*¹¹, in a more careful analysis of cells with *En(a-)* phenotype, used various radioactive probes to detect cell surface alterations and arrived at similar conclusions. These data, however, left some doubt, since absence of sialic acid and/or galactose from the glycoproteins would result in loss of just the structures which are required for detection by the techniques used. The attempt to label radioactively the protein backbone of glycophorin A and B by iodination gave equally ambiguous results, since glycophorin B could not be labelled in *En(a-)* cells despite the fact that this protein is labelled in normal cells and was found to be present in these cells by other techniques¹¹. In addition, the interpretation of data obtained by SDS-polyacrylamide gel electrophoresis is complicated by the lack or incomplete separation of glycophorin A and B from each other and other undefined glycoproteins^{21-24,26}, and is not entirely resolved by the use of other electrophoresis systems^{23,27}. Also these proteins tend to aggregate and to migrate electrophoretically at several positions, making quantitation difficult.

In order to overcome these problems we have developed a simple radioimmunoassay procedure which uses rabbit antibodies specific for a C-terminal determinant of glycophorin A. Since these antibodies react with the protein portion of glycophorin A exclusively, post-translational modification of the proteins on the N-terminal segment (which apparently is required for the expression of *MN* activity) or absence of glycosylation should not affect the assay. By using this assay the amount of glycophorin A for normal, unmodified human erythrocyte membranes was determined as 1.6% of the total membrane protein. Removal of sialic acid does not affect the results. Since the reaction of antibodies with glycophorin A can be specifically inhibited by small C-terminal peptides derived from glycophorin A (ref. 17 and unpublished results) and as membranes prepared from erythrocytes of other species, like guinea pig, sheep, or rat do not inhibit binding, no other membrane proteins seem to react or cross-react. Only chimpanzee cells were found to be reactive and contain about the same amount of glycophorin A or an analogue (unpublished) in accordance with the expression of *MN* activity in these cells³¹. When the variant cells listed in Table 3 were analysed in addition to membranes from one individual of the Finnish family heterozygous for *En(a-)*, the results shown in Fig. 3 were obtained. Three groups of cells can be distinguished: cells with no detectable glycophorin A, homozygous *En(a-)* G. W. or E. H.;

Table 3 Polyacrylamide gel electrophoresis and periodic acid-Schiff (PAS) staining of red cell membrane sialoglycoproteins* in genetic variants

Source of membranes	% of control membranes		
	Glycophorin A (PAS 1 & PAS 2)	Glycophorin B (PAS 3)	Total (PAS 1, 2, 3)
<i>M^NN</i>	98.4	101.1	97.2
<i>M^kM</i> (H.S.)	58.8	49.1	57.8
<i>M^kM</i> (F.Q.)	58.2	43.5	56.6
<i>En(a-)</i> (G.W.)	10.9	73	18.6
<i>En(a-)</i> (E.H.)	14.1	76.6	21.2
<i>Tn</i>	19.4	21	19.6

*Membranes were prepared by standard procedures³⁶ and dissolved immediately in buffer containing 5% sodium dodecyl sulphate. After incubation for 30 min at 37 °C the samples were applied to 5.6% polyacrylamide gels²⁶ and run until the tracking dye reached 8 cm. The gels were fixed, stained with PAS reagent, and scanned at 560 nm in 0.1% Na₂S₂O₅ in 0.01 N HCl. The peak areas were calculated by triangulation and normalised to equal protein loads. The data from three determinations obtained for variant cells are given in per cent as compared to normal cells for glycophorin A (PAS 1 and 2)²¹, glycophorin B (PAS 3)²¹, and total PAS-stainable protein recovered. For explanation of the variant cells see text.

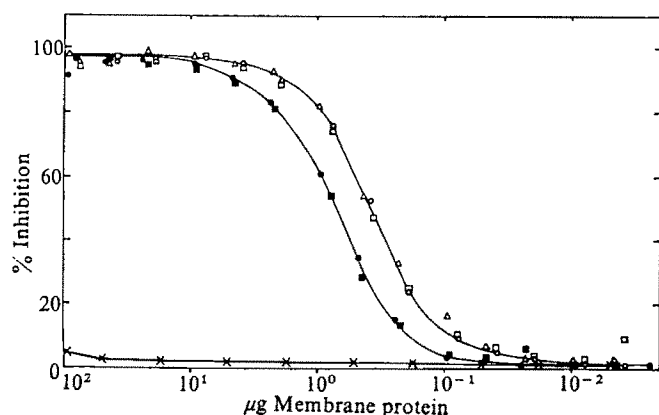


Fig. 3 Analysis of variants at the *MN* locus by quantitative radioimmunoassay-inhibition. Erythrocyte membranes from normal or variant cells (freshly prepared or kept frozen at -70°C) were dissolved in phosphate buffered saline, containing 0.02% NaN_3 and 0.15% Ammonyx-LO (lauryl dimethyl ammonium oxide) and were used in a radioimmuno-inhibition assay as described previously¹⁷. The protein concentration of the inhibitors was determined by amino acid analysis using norleucine as an internal standard or by the Lowry procedure with bovine serum albumin as the standard. Erythrocyte membranes from individuals with genotype M^kN (\square), M^kM (H.S. \bullet , F.Q. \blacksquare), $En(a-)$ homozygous (G.S. or E.H. \times) and two control individuals (\square , \triangle). *Tn* cells were indistinguishable from normal controls and $En(a-)$ heterozygous cells were identical to M^kM cells in this assay.

cells which contain 50% glycophorin A in comparison to normal, M^k heterozygotes or $En(a-)$ heterozygotes (not shown); and cells which are indistinguishable from normal controls, M^k heterozygotes or *Tn* cells (not shown).

Conclusions and perspectives

The *MNSs* locus determines two proteins, glycophorin A and glycophorin B, which are structurally related. The two alleles *M* and *N* determine a difference in amino acid sequence of glycophorin A, but this difference is restricted to two positions only. Serine and glycine are found in positions 1 and 5 of the amino acid sequence in glycophorin A^M and leucine and glutamic acid replace these amino acids in the corresponding positions in glycophorin A^N. This finding explains the heterogeneity previously observed in these two positions of the amino acid sequence of glycophorin A^{12,14,15}. Glycophorin B probably carries the antigenic determinant(s) which distinguish two allelic forms *S* and *s*, and was found to be identical in sequence with A^N in at least the first 23 residues regardless of the *MNSs* type of the cell membranes, from which the protein has been isolated. As the amino terminal peptide from glycophorin B reacts equally well with antibodies to glycophorin A^N, we conclude that the serological distinction between A^M and A^N resides within the amino terminal region of these proteins. This observation is consistent with the finding that red blood cells from *MM* homozygotes react with anti-*N* sera, particularly when treated with proteases, or are capable of removing antibodies from such sera in absorption experiments. The sialoglycoproteins determined by the *MNSs* locus are thus closely related, but not in the sense postulated by Springer^{7,32}, who proposed that the immunodeterminant structure of *M* and *N* is an oligosaccharide. The two structures were considered to be related since *N* was further postulated to be the biosynthetic precursor for *M*, which is generated by the addition of one sialic acid residue. According to this idea, *M* and *N* are genes which are responsible for post-translational modifications, but not structural genes as shown here. Our finding also does not favour the view that electrostatic interactions between ϵ -amino acid groups of lysine and carboxyl groups of sialic acid are important for a certain steric arrangement of the *M* and *N* specific structures³³. These ideas were based on the effects of modification of the protein by various compounds³⁴ and the changes of activity by removal of sialic acid or by amidation of the carboxyl group of sialic acid residues³⁵. The only two lysyl residues in the amino terminal glycosylated region are found in positions 18 and 30, and

glycosylation in the vicinity seems to interfere with proteolytic degradation by trypsin and chymotrypsin, probably due to steric hindrance or to certain conformational arrangements of the polypeptide backbone, which make these residues inaccessible. The lysyl residues, however, are separated by three and four amino acids, respectively, from oligosaccharide structures. Interaction between different subunits of glycophorin³⁶ could bring such residues in close proximity, but this does not seem to be likely, because small glycopeptides are only slightly less active than the intact protein in its isolated form. Alternative possibilities to explain the above findings are: a free amino group of the N-terminal serine or leucine may be important, as *M* or *N* activity can be restored after removal of the modifying groups³⁴; clustered oligosaccharides and sialic acids may impose certain restrictions to free mobility of the underlying polypeptide backbone. Removal of sialic acid could cause a change in the conformation of the protein, which is accompanied by loss of antigenic activity. It has been suggested that carbohydrates may influence or support certain protein conformations³⁷. More direct studies will be required to solve this question.

Serological studies in humans reveal abnormalities of expression of the *MNSs* antigens in certain rare instances³. Such abnormalities are correlated sometimes but not always with a considerable decrease in the amount of sialic acid associated with the plasma membrane. These observations are reminiscent of studies in mice in which strain differences in electrophoretic mobility and agglutinability are most easily explained by assuming one locus with only two alleles, *Eam*^h and *Eam*^l (ref. 38). The overall agreement between the distribution of the phenotypes in different crosses and the expectations from a simple diallelic system were sufficient to assume that one gene is of either unique or overriding importance in determining the surface charge of mouse erythrocytes. It was postulated that one gene either regulates the expression of a sialoglycoprotein directly or that it controls the quantity of a sialyl transferase.

In this study we have shown unambiguously that glycophorin A either can be totally absent from human erythrocytes or that the protein is altered so extensively that it can no longer be recognised as such; a total absence of glycophorin B is also possible²⁹, but no homozygous individual has been found (yet), who lacks both proteins. Loss of *MN* activity, however, is not necessarily associated with the absence of glycophorin A, as the two examples *Tn* and *M*^s show. For *Tn* cells a block in transfer of galactose is postulated^{28,42}. In *M*^s cells, as well as possibly in other variant forms³, the alteration seems to be much more subtle.

The absence of glycophorin A or B is not apparently associated with disease and to our knowledge no functional or other abnormalities have been described involving cell or membrane³⁹. Previous speculations on the role of various segments of these sialoglycoproteins for membrane organisation and function for the mature red cell membrane thus do not seem justified²². These cell surface molecules may be more important during earlier stages of differentiation. A genetic event involving cells committed to the erythrocytic series is expected to result in abnormal erythrocytes exclusively. We do not know when glycophorins are synthesised first and whether their synthesis is restricted to cells of the erythrocytic series. Possibly even in variant cells these proteins are made during the early phases of haematopoiesis, but are lost during later stages of differentiation. It is certain, however, that the absence of glycophorins alone does not describe all the alterations of the membrane which do occur in these variant cells and which involve other proteins and/or lipid components of the membrane¹¹.

We thank Drs Issitt, Lalezari, Leikola, Luner, Miller and Metaxas for samples of variant blood and Mr B. Proctor for technical assistance. Research support was obtained from the Anna Fuller Fund and the American Cancer Society BC-102C.

Received 25 August; accepted 2 December 1977.

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letters to nature

Revised estimate of gravitational radiation from Crab and Vela pulsars

PROGRESS in the development of ultra-high-Q dielectric crystals (Bagdasarov *et al.* unpublished data) has caused experimenters in the field of general relativity to look for rotation-induced gravitational radiation from pulsars (D. H. Douglass, unpublished data). If one could control the frequency of oscillation of a high-Q crystal, keeping it in phase with the electromagnetic signals observed from a pulsar, one might hope to absorb a measurable amount of (quadrupole) gravitational radiation at twice the pulsar frequency. Press and Thorne¹ in 1972 estimated that the gravitational waves from the Crab pulsar would produce a dimensionless strain in a detector on Earth of $h \sim 10^{-26}$ to 10^{-28} , and that other pulsars would be several orders of magnitude fainter. Additional observational data, and progress in pulsar models during the past five years, make a new estimate desirable. I report here that the amplitude of the gravitational waves from the Crab pulsar (PSR0531+21) is likely to be within 2 orders of magnitude of 10^{-27} , but that the Vela pulsar (PSR0833-45) is likely to produce waves of amplitude a factor 10 to 100 larger.

The waves are produced by the rotation of mass asymmetries in the neutron star. Of all conceivable mass asymmetries, conventional pulsar theory^{2,3} points to one as the most likely

to dominate the radiation: the neutron star must be rotationally flattened with oblateness $\epsilon_0 \equiv (\text{equatorial radius} - \text{polar radius})/(\text{mean radius})$. If the star were to rotate about its polar axis of symmetry, it would not radiate gravitationally. But, the observed 'restless' behaviour of the Crab and Vela rotation periods P (refs 2, 4) makes it likely⁵⁻⁸ that the rotation axis and the symmetry axis are misaligned by a small angle θ_w . Such a misalignment would produce a 'Chandler wobble' in the star's rotation⁶, and the wobble-induced strains would produce microquakes that provide an attractive and successful explanation^{5,7} for the observed 'restlessness'. The misalignment would also cause a fraction $\epsilon \equiv \epsilon_0 \theta_w$ (for small θ_w) of the star's moment of inertia I to radiate as a time-changing quadrupole moment. The resulting luminosity in gravitational quadrupole radiation would be¹

$$L_{\text{GW}} = \frac{32G}{5c^5} \epsilon^2 I^2 \left(\frac{2\pi}{P} \right)^6 \approx 5 \times 10^{32} \text{ erg s}^{-1} \left(\frac{\epsilon}{2 \times 10^{-8}} \right)^2 \left(\frac{I}{4 \times 10^{44} \text{ g cm}^2} \right)^2 \left(\frac{P}{0.033 \text{ s}} \right)^{-6}$$

Of the crucial parameters, P is determined by radio and optical observations⁴ to great precision. The distances to the pulsars are less well known, but the association with supernova remnants yields distance estimates of 2,000 pc and 500 pc for the Crab and Vela respectively, good to within about 25%^{4,9}. Neutron-star moments of inertia depend both upon the assumed mass of the star and upon the equation of state of matter at high densities. But because the stellar radius tends to decrease as mass increases, I is somewhat buffered; early equations of state² gave $7 \times 10^{43} \lesssim I \lesssim 7 \times 10^{44} \text{ g cm}^2$, but more recent work¹⁰ suggests $3 \times 10^{44} \lesssim I \lesssim 3 \times 10^{45} \text{ g cm}^2$. Within this range, the original Pines and Shaham⁵ 'crustquake' explanation of the Crab pulsar glitches agrees with the spindown power output ($I\dot{\Omega}$) required to drive the nebula^{2,11} and implies $I \sim 4 \times 10^{44} \text{ g cm}^2$ (though Pandharipande, Pines and Smith (PPS) for their 1.33 M_\odot Crab model¹⁰ find $I \sim 2 \times 10^{45} \text{ g cm}^2$). The Vela pulsar, which has experienced at least three 'giant' speedups within the past 8 yr (refs 4, 12) is modelled as a more massive, solid-core object¹⁰ with the moment of inertia¹⁰ $I \sim 2$ to $3 \times 10^{45} \text{ g cm}^2$.

The oblateness of the star, ϵ_0 , is predicted by standard star-quake theory and by calculations of the critical strain which the crust (for the Crab) or the core (for Vela) can withstand before

Table 1 Estimated pulsar gravitational radiation			
	Standard Crab	PPS ¹⁰ Crab	Vela
$P(\text{s})$	0.033	0.033	0.089
$\omega_{\text{GW}}(\text{s}^{-1})$	380	380	140
$R(\text{pc})$	2,000	2,000	500
$I(\text{g cm}^2)$	4×10^{44}	2×10^{45}	2×10^{45}
ϵ_0	2×10^{-4}	4×10^{-4}	3×10^{-3}
θ_w	10^{-2}	10^{-2}	10^{-2}
$L_{\text{GW}}(\text{erg s}^{-1})$			
max	5×10^{32}	4×10^{34}	1×10^{34}
min	1×10^{35}	1×10^{37}	2×10^{37}
$\mathcal{F}(\text{erg cm}^{-2} \text{ s}^{-1})$			
max	1×10^{-12}	9×10^{-11}	4×10^{-10}
min	4×10^{-10}	4×10^{-8}	1×10^{-6}
h			
max	1×10^{-27}	9×10^{-27}	5×10^{-26}
min	2×10^{-28}	2×10^{-26}	3×10^{-24}
	2×10^{-29}	3×10^{-28}	1×10^{-27}

fracturing. The resulting values are, for the Crab^{2,6,8}, $\varepsilon_0 = (1-2) \times 10^{-4}$ (though the PPS 1.33 M_\odot model¹⁰ has $\varepsilon_0 \approx 3.5 \times 10^{-4}$), and for Vela^{8,10,13}, $\varepsilon_0 \sim 10^{-2}$ to 10^{-3} . The fraction of this oblateness which is effective in producing gravitational radiation is θ_w , the wobble angle. The microquake theories suggest that θ_w is limited by starquakes to values of the order of 0.1 radians (refs 5, 7, 8); however, the lack of observed wobble⁴ (and in particular, the constancy and sharpness of the optical Crab light curve) suggests that 0.1 be taken as an extreme upper bound, and that θ_w probably lies between 10^{-3} and 10^{-2} radians. The wobble question is the most uncertain part of this analysis (see ref. 6 for comments and references).

The most probable values for the vital parameters in determining the gravitational luminosity are summarised in Table 1, for the standard Crab model, the PPS¹⁰ (stiff equation of state) 1.33 M_\odot Crab, and for Vela. The resulting gravitational wave luminosity L_{GW} , the energy flux at Earth $\mathcal{F} \equiv L_{GW}/4\pi R^2$, and the wave amplitude (strain, or metric perturbation)¹⁴ $h \equiv (16\pi G\mathcal{F}/c^3\omega^2\tau)^{1/2}$, are given together with their probable ranges using the parameter estimates described above. The errors have been added coherently, not by quadrature, so the range covered is rather large; most of the uncertainty comes from the uncertainty in θ_w .

It is possible, of course, that something is radically wrong with the above assumptions. On the other hand, if internal toroidal magnetic fields exist with $B \gtrsim 10^{15}$ G (refs 3, 6), they could produce an oblateness $\varepsilon_0 \sim 10^{-35} (B/1 \text{ G})^2$ comparable with or larger than the fluid oblateness. If the protons in the neutron star form a type-2 superconductor^{15,16} with critical field $H_{c1} = (4 \text{ to } 8) \times 10^{14}$ G, then in the low-flux-density limit ($B \ll H_{c1}$) $\varepsilon_0 \sim 10^{-35} B H_{c1}$ and so smaller internal fields may begin to cause a significant oblateness. In both cases, the internal fields may tend to align themselves perpendicular to the spin axis of the star¹⁶ (effectively $\theta_w = 90^\circ$) and would thus be maximally efficient in producing gravitational radiation. An internal field of 10^{15} G would make a wave of amplitude $h \sim 5 \times 10^{-27}$ from the Crab's distance; a field of 10^{12} G in a type-2 superconductor would produce $h \sim 2 \times 10^{-30}$. So it is improbable that h is much less than the minimum estimates (2×10^{-29} for the Crab, and 1×10^{-27} for Vela) which starquake theory suggests.

The wave amplitude h could, however, be larger. Mountains or other local inhomogeneities in the crust or core could conceivably produce a net nonaxisymmetric oblateness of the same order of magnitude as the materials' shearing strengths^{2,10}, several orders of magnitude larger than the starquake models predict. An extreme upper limit on L_{GW} can be set by requiring that gravitational radiation account for the entire observed slowdown of the pulsars. That limit yields⁴ for the Crab, $L_{GW} \lesssim 2 \times 10^{38} \text{ erg s}^{-1}$, $\mathcal{F} \lesssim 7 \times 10^{-7} \text{ erg cm}^{-2} \text{ s}^{-1}$, $h \lesssim 8 \times 10^{-25}$ and for Vela, $L_{GW} \lesssim 2 \times 10^{37} \text{ erg s}^{-1}$, $\mathcal{F} \lesssim 1 \times 10^{-6} \text{ erg cm}^{-2} \text{ s}^{-1}$, $h \lesssim 3 \times 10^{-24}$, which coincidentally agrees with the upper-bound estimate for Vela on the basis of maximum credible oblateness (Table 1).

To detect the quadrupole radiation at frequencies ω_{GW} of 380 s^{-1} for the Crab and 140 s^{-1} for Vela, some workers envision using large monocrystals, probably of sapphire or silicon (Bagdasarov *et al.*, and Douglass, unpublished data). For crystals of effective length l and effective mass m , the change in amplitude of oscillation due to absorption of gravitational waves in phase with the crystal's oscillation during a measurement time τ is (assuming $\tau \ll \tau^*$, the damping time):

$$\Delta X_{GW} = \tau \omega h / 2$$

Brownian motion at temperature T in a crystal with damping time τ^* ($\tau^* \equiv 2Q/\omega$) produces an amplitude change

$$\langle \Delta X_{Brwn}^2 \rangle^{1/2} = (2\tau k T / m \omega^2 \tau^*)^{1/2}$$

where k is Boltzmann's constant.

For a reasonable signal-to-noise ratio¹⁷ of 10, the minimum detectable wave has amplitude

$$h \gtrsim 10 \left[\frac{2kT}{mQ\omega^3 l^2 \tau} \right]^{1/2} \approx 7 \times 10^{-28} \left(\frac{T}{10^{-3} \text{ K}} \right)^{1/2} \cdot \left(\frac{10^5 \text{ g}}{m} \right)^{1/2} \cdot \left(\frac{10^{14}}{Q} \right)^{1/2} \cdot \left(\frac{380 \text{ s}^{-1}}{\omega_{GW}} \right)^{3/2} \cdot \left(\frac{10^2 \text{ cm}}{l} \right) \cdot \left(\frac{10^7 \text{ s}}{\tau} \right)^{1/2}$$

It is straightforward to verify that detection of this signal would not require a 'quantum non-demolition sensor'^{17,18}, though the construction of the required sensor would be a nontrivial task. The minimum detectable wave amplitude for Vela is a factor of 4 higher, due to its lower frequency. The values of T , m , Q , l , and τ assumed above are not unreasonable goals for the next 5–10 yr of experimental effort¹⁹. It thus seems that, if the Crab or Vela pulsars are as strong as the best estimates indicate, they may be borderline-detectable by gravitational astronomers within the 1980s.

I thank Carlton M. Caves, Peter Goldreich, Kip S. Thorne and David Douglass for helpful comments on this subject.

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Received 12 September; accepted 1 December 1977.

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Distance limit for a class of model γ -ray burst sources

THE observed time structures of γ -ray bursts of the type reported by Klebesadel *et al.*¹ place an upper limit $l \approx 10^9 \text{ cm}$ on the typical size of the burst source regions². If the sources were too far away and isotropically radiating then the observations would require such a high density of photons that the sources would be optically thick in the MeV region simply due to γ - γ -pair production. These considerations do not apply directly to highly beamed sources or relativistically expanding sources. I point out here that MeV photons have actually been observed in bursts, and that this means that non-relativistic sources cannot be further away than a few kpc from the Sun and therefore must be galactic.

Some of the available spectral burst data^{3–8} show a significant flux of photons in the $> 1 \text{ MeV}$ range^{3–5,8}. The 27 April 1972, event observed by Apollo 16 (ref. 3) shows at higher energies a power law spectrum with a possible line feature around 4 MeV. However, power law spectra and lines cannot escape with significant intensity from homogeneous optically thick sources, and if the lines were generated in different spatial regions that are optically thin it would seem to be difficult to get the energy into

these regions to excite MeV lines and nothing else. Another possibility would be to generate the burst in a thin shell surrounding the volume of radius l . But that does not significantly decrease the self-absorption of the source, as shown below.

In case of rapidly varying thermal radiation from an optically thick source it is not easy to construct a spectrum that is a power law at high energies when integrated over the burst duration. A cooling blackbody can account for some lower energy features of the burst spectrum⁹, but not for the high energy tail.

For most events there is insufficient information available on the time structure of the burst around 1 MeV. However, the 18 December 1972 event—although it was not observed at 1 MeV—showed no rapid spectral changes on the time scale of the fast sub-bursts⁷, and a generally increasing hardness near the beginning of the burst. These data suggest that the high energy fast time structure may not be significantly different from the lower energy time structure.

The significance of photon-photon pair production in astrophysical situations has been investigated for a variety of cases. Nikishov¹⁰, and Fazio and Stecker¹¹ considered the absorption of very high energy photons by thermal photons in intergalactic space. Jelley¹² investigated that same mechanism for quasars and other radio sources. McBreen¹³, and Stecker and Tsuruta¹⁴ applied these considerations to the Crab pulsar NPO532, and Rengarajan¹⁵ considered the escape of γ rays from hot neutron star surfaces. In a more general assessment Herterich¹⁶ showed that intense X-ray sources are optically thick for γ rays above a few MeV and therefore cannot be high energy γ -ray sources.

To estimate the optical depth of a homogeneous, isotropic radiation field we have used the formulae as used earlier by Nikishov¹⁰, Jauch and Rohrlich¹⁸, and Gould and Schröder¹⁷. For simplicity we assume that all photon-photon encounters take place at $\theta = 90^\circ$ between the momentum vectors of two interacting photons. The threshold energy ε_{th} of an ambient photon for photon-photon pair production, interacting with a test photon of energy E , is

$$\varepsilon_{th} = (2m^2c^4)/E(1 - \cos\theta) \quad (1)$$

Here mc^2 is the electron rest energy. The cross section $\sigma_{\gamma\gamma}$ has a maximum of $1.7 \times 10^{-25} \text{ cm}^2$ at $\varepsilon_0 = 2\varepsilon_{th}$.

For a typical source radius l and ambient photon density $n \text{ cm}^{-3}$, the optical depth for the test photon is typically

$$\tau(E) = l \int_{\varepsilon_{th}}^{\infty} n(\varepsilon) \sigma_{\gamma\gamma}(E, \varepsilon) d\varepsilon \quad (2)$$

With the observed photon number flux $F(\varepsilon) \text{ cm}^{-2} \text{ s}^{-1} \text{ keV}^{-1}$ the spatial density of photons on the surface of the source is $n = (F/c)(D^2/l^2)$ where D is the distance of the source; hence

$$D(E) = \left(\frac{\tau l c}{\int_{\varepsilon_{th}}^{\infty} F(\varepsilon) \sigma(E, \varepsilon) d\varepsilon} \right)^{1/2} \quad (3)$$

For integration we make two alternate assumptions for the spectral shape of the flux $F(\varepsilon) \text{ cm}^{-2} \text{ s}^{-1} \text{ keV}^{-1}$. We consider (a) a composite spectrum⁴

$$\begin{aligned} & \frac{F^{(a)} \text{ cm}^{-2} \text{ s}^{-1} \text{ keV}^{-1}}{(\text{erg cm}^{-2} \text{ s}^{-1})} \\ &= 1.6 \times 10^4 \exp(-\varepsilon/150 \text{ keV}) \quad (\varepsilon \leq 375 \text{ keV}) \\ &= 3.9 \times 10^9 (\varepsilon/\text{keV})^{-2.5} \quad (\varepsilon > 375 \text{ keV}) \end{aligned}$$

and (b) for comparison we consider a pure power law

$$\frac{F^{(b)} \text{ cm}^{-2} \text{ s}^{-1} \text{ keV}^{-1}}{(\text{erg cm}^{-2} \text{ s}^{-1})} = 1.9 \times 10^8 (\varepsilon/\text{keV})^{-2}$$

This is the photon number flux normalised to a burst of integrated energy flux of $1 \text{ erg cm}^{-2} \text{ s}^{-1}$. For normalisation of case (b) we

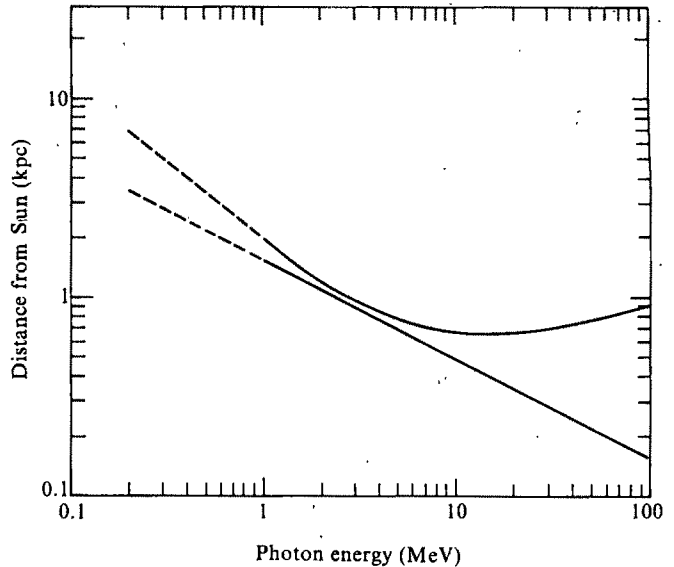


Fig. 1 Distance where source of $3 \times 10^{-5} \text{ erg cm}^{-2} \text{ s}^{-1}$ burst is optically thick, $\tau = 1$. Homogeneous isotropically radiating burst sources are optically thick at the abscissa energy if assumed to be further away from the Sun than indicated on the ordinate. The two photon number spectra of bursts (upper curve for the composite spectrum) have been assumed without cutoffs.

have assumed that 70% of the usually quoted energy of the burst is found between 100 keV and 1,000 keV.

As an example we take a typical burst of size $10^{-4} \text{ erg cm}^{-2} \text{ s}^{-1}$. The actual energy flux of such a burst is around $3 \times 10^{-5} \text{ erg cm}^{-2} \text{ s}^{-1}$ with wide variations. We assume now that the optical depth of the source region should be $\tau < 1$ in order to assure the escape of the test photon. With this we arrive at Fig. 1. Since a fair number of events have been detected near 1 MeV (refs 3–5, 8), and all show deviations from an exponential spectrum, we take the $\tau = 1$ distance derived for 1 MeV for the upper limit of the distance to a $10^{-4} \text{ erg cm}^{-2}$ homogeneous isotropic burst source, which is $D \approx 2 \text{ kpc}$ according to Fig. 1. Thus this type of burst source must be galactic. The photon number flux fluctuates greatly during the burst, and the actual momentary fluxes are often significantly greater than the average fluxes. So our upper limit should be rather generous.

This distance limit was estimated for $l = 10^9 \text{ cm}$ sources which are isotropically radiating with initial photon density constant throughout the source volume and the same as on its surface. If the radiation were to emerge only from a surface around the source, but each surface element were radiating isotropically, then near the source the photon density would still be quite high, but the average angle of encounter would be less than 90° . A similar situation was considered previously¹⁹, and for our case we find that the $\tau = 1$ distance would be insignificantly larger than our upper limit. Also, if the high energy radiation of the event of 27 April 1972 were a 4.4-MeV carbon line feature with a typical time scale of about 5 s and intensity $10^{-1} \text{ s}^{-1} \text{ cm}^{-2}$, then the typical source radius would be about 10^{11} cm , and the resulting $\tau = 1$ distance would be about $3 \times 10^5 \text{ pc}$. Thus even this very extreme assumption would keep the source well within the local group of galaxies.

Within 2 kpc of the Sun there is about 1% of the galactic mass; this holds for either all matter²⁰ or the extreme population I as represented by the hydrogen disk²¹. If the bursts come from objects distributed throughout our Galaxy similar to either one of the two classes of objects then bursts of size $10^{-4} \text{ erg cm}^{-2}$ come from only about 1/100 of the Galaxy. Assuming the widely used standard candle hypothesis where the burst size is inversely proportional to the square of the source distance, the frequency of bursts of size $\geq S$ is at the same time a lower limit to the frequency of occurrences of bursts at distance $\leq D(S)$. Since the frequency of detections of $10^{-4} \text{ erg cm}^{-2}$ bursts is about three per year (refs

22, 23) the upper limit to the distance leads to an upper limit of the average intrinsic luminosity of $\approx 5 \times 10^{40}$ erg and to a lower limit of about 300 burst occurrences per year throughout our Galaxy.

This means that unless very special radiation mechanisms are at work occurrences that are unique in the history of an individual star cannot account for the majority of the bursts that are being detected. The rate of star formation in our Galaxy is at present about two to three solar masses per year²⁴ and supposedly has been constant for the last few times 10^9 yr. We assume for the sake of this argument that this means two to three stars per year. It is difficult to see how the rate of irreversible star transformations could be two orders of magnitude higher than this rate at the present epoch. Nova explosions also have been suggested as γ -ray burst sources. The rate of optical novae per year has been estimated to be about 30 to 50 in our Galaxy^{25,26}, and 25 to 30 in M31 (refs 27, 28). These numbers would be, considering the usual uncertainty of numbers in astrophysics, marginally compatible with our number of burst occurrences. However, considering the high degree of isotropy of the detected burst directions^{22,29} we feel we can also exclude novae as the major contributors to γ -ray bursts. Therefore, we suggest tentatively that the γ -ray bursts that have been detected are galactic, but are in the majority of the cases not connected with unique irreversible star transformations, and also it is unlikely that they are connected with galactic novae.

In the case of beamed sources the threshold energy (1) depends on the opening half angle $\Gamma/2$ of the beam. Using the same arguments as Stecker and Tsuruta¹⁴ we assume $\theta \approx 2^{1/2} \Gamma/2$. Assuming that 5 MeV photons have been observed^{3,5} we set $\epsilon_{th} \approx 2.5$ MeV and from this with (1) we derive $\Gamma/2 \approx 12^\circ$. With (3) we find that such a source should be optically thick ($\tau = 1$) at 5 MeV if placed further than 6 kpc from the Sun. The galactic fraction of matter within this distance is about 1/10 of the total, and the solid angle covered by the $\Gamma/2 = 12^\circ$ burst is $1/8$ sr, so that in the same way as above we arrive at a minimum number of burst occurrences throughout the galaxy of $8 \times 4\pi \times 10^3 \approx 3,000$ yr⁻¹. Hypothetical beams narrower than 12° cannot be assessed in this way, because the energy where absorption can become important ($\leq 2 \epsilon_{th}$) is not covered by observations, yet. Thus for not too narrow beams the conclusions are essentially the same as above.

I emphasise here that the conclusions reached depend entirely on observed parameters. No specific assumption had to be made about particles or fields within the sources. Generally, particles or fields can also serve as absorbing agents so that their presence can only increase the optical depth of the source and therefore put even more severe constraints on the distance of the sources.

I thank F. W. Stecker, R. Ramaty and T. L. Cline for helpful discussions. I am an NAS/NRC Resident Research Associate.

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Received 15 July; accepted 23 November 1977.

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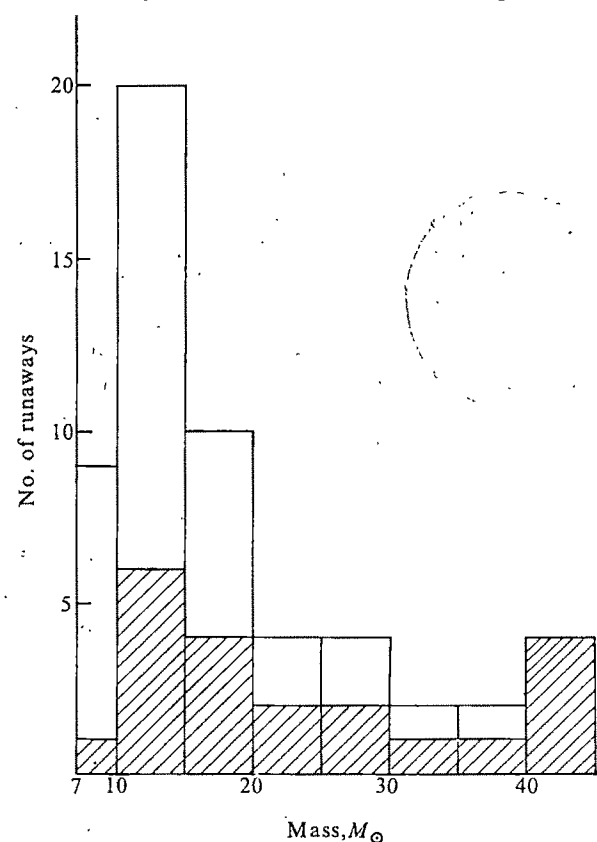
Runaway stars as witnesses to supernova explosions

THE hypothesis that single runaway stars are released from close binaries in which the companion star explodes as a carbon detonation supernova and is totally disrupted¹ is proposed here. This is contrary to the earlier belief² that at the time of the explosion the more evolved star is the less massive so that the supernova explosion which leaves behind a relativistic star core does not disrupt the binary³ but accelerates it to a velocity of a few tens of km s⁻¹ (ref. 4). The binary consisting of a normal OB star and an inactive compact star could be detected as a runaway till it shows itself as an X-ray source.

From the stars that can explode as supernova, it is only the lower mass stars that burn carbon explosively⁵. Therefore if the above hypothesis is correct, lower mass runaways should be predominantly single, while the higher mass runaways should be predominantly binaries containing inactive compact stars. This is shown to be the case in Fig. 1 (based on data from ref. 6). Of the 55 runaways (peculiar velocities ≥ 40 km s⁻¹) listed, 29 have masses $< 15 M_\odot$ out of which only 7 (24%) are deduced to be binaries on the basis of radial velocity variations. On the other hand, out of 26 runaways with masses $\geq 15 M_\odot$, 14 (54%) are deduced to be binaries.

For a supernova explosion to take place in a close binary, the primary (the 'first' star) should start with a mass $\geq 15 M_\odot$. If the

Fig. 1 Mass distribution of runaways. The shaded area refers to the distribution of runaway binaries containing inactive compact stars. Runaways with masses $\geq 40 M_\odot$ are clubbed together.



second star is to collect the matter being transferred from the first star it should have a main sequence mass of about $4 M_{\odot}$ (ref. 3), because if the mass ratio is smaller than 0.3, mass transfer to the second star would make it thermally unstable, leading to the formation of a contact binary and large mass loss from the system⁷. If all the mass lost by the first star is collected by the second star, there must be a lower limit on the mass of a single runaway star. Assuming that the main sequence masses of the components were $15 M_{\odot}$ and $4 M_{\odot}$ and that the mass transfer left the first star as a helium star of mass $5 M_{\odot}$ whose supernova explosion disrupted the binary, the minimum mass a runaway could have is $14 M_{\odot}$. The fact that there are runaways with masses as small as $7 M_{\odot}$ (ref. 6) proves that during mass transfer phase, about 50% of the mass being transferred is lost from the system. A similar mass loss has been invoked⁸ to obtain a better quantitative agreement between theory and observations for Algol-type systems.

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Received 28 June; accepted 20 October 1977.

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Azimuthal brightness variation of Saturn's ring-A and size of particles

OBSERVATIONS of the brightness of ring A of Saturn, indicate that it is not constant in azimuth. This was discovered by Camichel¹, and later studied by me². Reitsema *et al.*³ and Lumme and Irvine⁴, have confirmed these observations. The A ring is fainter in quadrants following conjunction of the particles with the Earth-Saturn line, and brighter in quadrants preceding conjunction. No azimuthal brightness variation of this type is found for the B-ring⁵. Because these observations do not show any time dependence, the effect is stationary. We will call this, fact A. A possible explanation for it has been given by Colombo *et al.*⁶. Here we give an alternative explanation for fact A, which would be valid if the particles in ring A are large, which we will show is indeed the case, using a recent measurement¹¹ of the total mass of the rings.

This observational fact implies four conditions: (1) the light showing the variation has to come from the surface of the particles (the amplitude of this variation is around 30%); (2) the particles are in synchronous rotation; (3) rotations around the planet-particle axis are not allowed; (4) the albedo of the particles changes around them, or they are elongated and tilted with respect to the orbital radius. Note that all these conditions must be satisfied. If any were not, fact A would not apply. Condition (1) rules out the bright-cloud model of Pollack⁶ for ring A. That the particles are in synchronous rotation is not a surprise, as most of the satellites of Saturn are in such a state⁷. Condition (3) implies that collisions are not capable of turning the particles around, or that they are not important today. Finally, condition (4) presents two alternatives: an albedo change over the particles, or particles with their major axis not pointed exactly to Saturn, but inclined to it. A possible explanation of fact A based on this last possibility, has been given by Colombo *et al.*⁸, using a mechanism of travelling waves related to the one that produces the spiral arms in galaxies. Another possibility based on the first alternative (an albedo variation over the particles) implies that collisions have been important in Saturn's rings in the past, and it seems to us a much simpler explanation for fact A.

To understand this mechanism, we will use Fig. 1 which shows two particles of the rings seen from above, which are about to

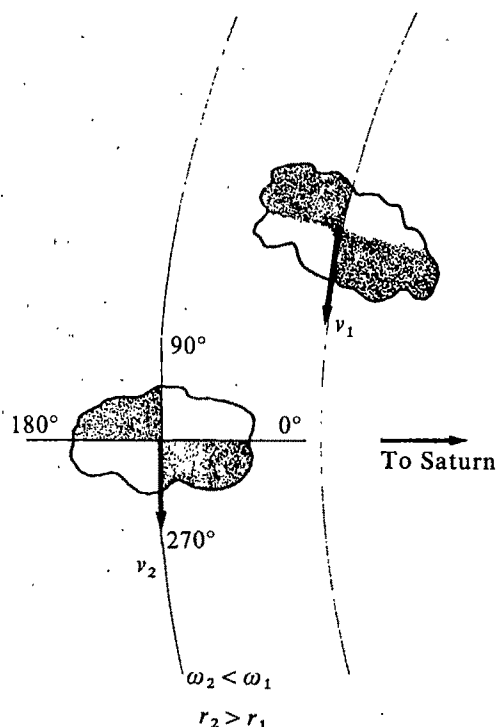


Fig. 1 Two particles about to collide in the rings. As particle 1 is nearer to the planet, it moves faster. Then collisions can occur only in the third quadrant (180° to 270°) of the first particle, or in the first quadrant (0° to 90°) of the second. There is no way in which the second and fourth quadrants of the two particles can be reached, if the particles preserve their relative radial positions. Note that the collisional regions are bright. This configuration is consistent with the azimuthal brightness variation observed in ring A.

collide. Particle 1 moves faster than particle 2, since $r_1 < r_2$, and so 1 overtakes 2. It is clear then that if we use the system of coordinates drawn on particle 2, collisions can occur only in the third quadrant ($180^\circ < \phi < 270^\circ$) of the first particle, and on the first quadrant ($0^\circ < \phi < 90^\circ$) of the second particle. There is no way in which the other quadrants can be reached. Thus we would expect some qualitative changes on the surface properties of the particles due to the effects of collisions in these two quadrants. Observationally, fact A shows that the collisions produce bright regions.

The above explanation is valid only if each particle preserves its radial positions with respect to others. Franklin and Colombo⁹ have shown that a particle at the centre of ring A is displaced by $\sim \Delta R = \pm 147$ m in the radial direction due to perturbations by the satellites. The rate of precession of the orbits of the different particles in the rings due to the Sun and other Saturnian satellites, is a function of the radial distance to the planet. Contiguous orbits will have different rates. Secularly the radial displacements for two nearby particles will not be in phase, but their values will still be given by ΔR . So for a particle to preserve its position with respect to others, its size has to be of the same order of magnitude or larger than ΔR . If this were not the case, collisions would be allowed in all quadrants and no azimuthal variation would be produced. Thus the diameter of the particles is $D > 2\Delta R \approx 290$ m. This value again rules out the bright-cloud model of Pollack⁶ for ring A, since it requires $D \approx 10$ cm.

Additional evidence for large particles can be derived from a paper by McLaughlin and Talbot¹¹. They have obtained the total mass of the rings, from the observed secular apsidal and nodal rates of the inner satellites. They found $M_{\text{rings}} = (6.2 \pm 2.4) \times 10^{-6} M_{\text{Saturn}}$, for a rotational period of Saturn of 10 h 26 min. If a period of 10 h 14 min is adopted², their value is changed only slightly to $(5.9 \pm 2.4) \times 10^{-6} M_{\text{Saturn}}$. I have pointed out previously¹⁰ that the mass of the rings and the mean particle size are linearly related, and knowing one of them

permits the evaluation of the other. I also presented a functional relationship, for mean densities of 1.0, 2.0 and 5.0 g cm⁻³. Using the later value of the mass, we obtain mean radii of the particles of $\bar{r} = 1.9, 1.0$ and 0.5 Km, for the different densities, respectively. It is hard to escape from this conclusion, because this result is based on well known determinations of the optical thickness of the rings^{5,10}, and is insensitive to the ring model adopted.

A possible stable configuration in which the particles would preserve their radial positions could be if the particles move in an orderly fashion in smaller rings, of separation $s \gtrsim D \gtrsim 2\Delta R$ (see Fig. 2). There is some evidence that this BIAS model (beads in a string) could be correct.

Jeffreys¹² had shown that a multilayer of particles rotating around a massive primary, would transfer angular momentum inward and outward, until the ring becomes one particle thick, and the particles are separated radially by a distance equal to their diameters. The transfer of angular momentum then ceases. He derived an approximated formula for the time to reach this state: $t \simeq 10^6 \text{ yr}/D \text{ (cm)}$. This time is very short even for small particles. Presumably Saturn's rings should be in this state, and thus collision should be rare between radially neighbouring particles, in agreement with condition (3).

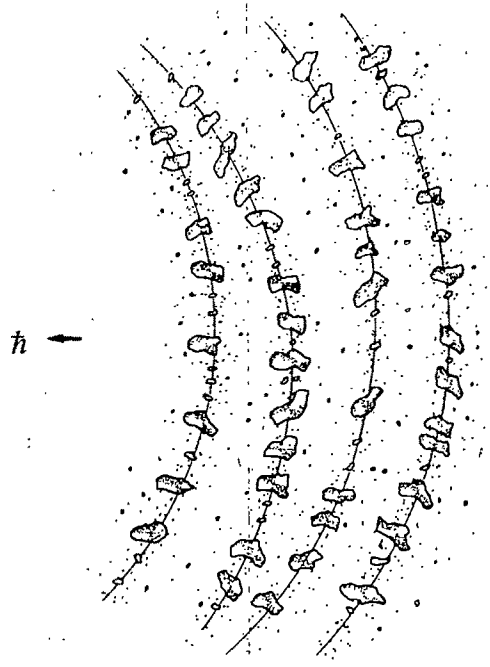


Fig. 2 BIAS configuration proposed for ring A. In this model particles move orderly in concentric sub-rings. The particles are slightly elongated, and point toward Saturn maintaining synchronous rotation. There is a fine envelope of smaller particles surrounding the rings. These would help to damp oscillations and add to the stability of the configuration. Only the large particles show an azimuthal effect.

Once the particles are in a monolayer, current theories of jet-streams^{13,17} indicate that narrow rings could be generated. In trying to prove this result, Baxter and Thompson¹⁴ have taken a population of disks in a plane, moving in keplerian orbits around a primary. Disks were taken due to mathematical simplicity. The Boltzmann-like kinetic equation for the development of the particle distribution function could be expanded as a Fokker-Planck equation for the diffusion of that function. If the elasticity of the collision were small, the diffusion coefficient would be negative, indicating that regions of enhanced density would be formed. However, a rather low value of the elasticity was found necessary for this to happen. Due to the neglect of several important physical processes, their value must be considered as a lower limit. A realistic model should include besides collisions, sliding friction between the surfaces, work done to

displace the particle from its Saturn orientation, and a possible cloud of gas and dust around the particles, that could damp all oscillations. For example, the recently discovered linear markings on the martian satellite Phobos¹⁵, could be interpreted as evidence of a sliding collision with another celestial body. From the depth of these markings it is clear that sliding friction could have been rather substantial. Incidentally, the apparition of narrow rings of enhanced density was also found for the case of accretion disks surrounding black holes¹⁶.

Alfvén¹⁷ has considered the case of several apples inside a spacecraft, rotating around a planet. He found that the apples would be found neatly located at the centre of the spacecraft, in a line parallel to the orbit. In this case the focusing is produced by the walls of the spacecraft. Particles in Saturn's rings are not inside a spacecraft, but the surrounding particles could conceivably produce the same effect.

Perhaps the best evidence in favour of a BIAS model is not theoretical but observational. Such evidence lies with the discovery of narrow rings around Uranus^{18,19}, situated $\sim 40,000$ km from the planet. At the distance of Uranus, the size of the occulted star was around 4 km, so a large resolution was not possible. But from the duration of some of the events (~ 0.7 s) the conclusion is that these rings are very narrow, and cannot be wider than a few kilometres^{18,19}. The particles composing this ring have then to be smaller than a few kilometres. Note the coincidence of this value with that for the particles in Saturn's rings. This is the best evidence that narrow rings are formed in the Solar System, and could have been formed in Saturn's rings as suggested by fact A.

In conclusion, large particles for the rings have several advantages: first, they explain fact A if particles are in a BIAS configuration; second, they explain the shift in position of the outer boundary of ring B, found by Franklin *et al.*⁹; third, it is the only way of obtaining a large mass for the rings¹⁰, as found by McLaughlin and Talbot¹¹.

This work was supported by grants from CONICIT of the Government of Venezuela, and CDCH of the University of the Andes.

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Received 19 September; 22 November 1977.

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Observations of strong wind shear using pulse compression radar

THE occurrence of layers of strong wind shear is interesting because of their relationship to turbulence and because of their effect on aircraft, rockets and missiles crossing them. The most extensive source of data on wind shear is from ascending balloon-borne targets but these provide individual profiles of uncertain representativeness and often lack vertical resolution. Measurements from aircraft provide more detailed data. Other sources of high resolution data being used increasingly are

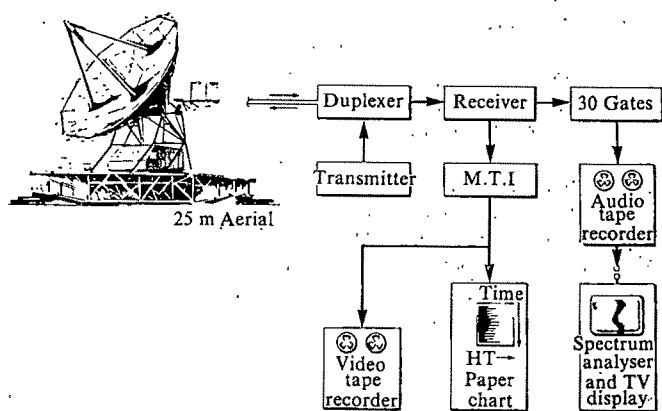


Fig. 1 Forms of data output from the Defford radar.

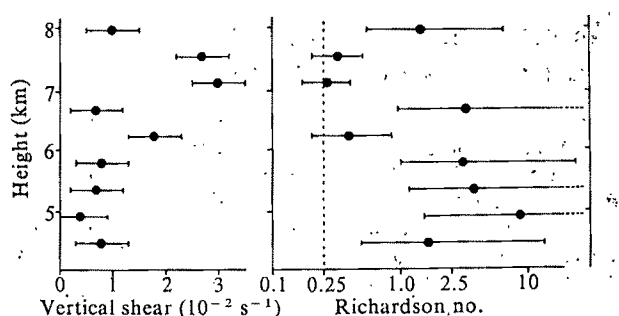
remote probing techniques capable of measuring the Doppler shift from windborne natural targets, such as, cloud or dust particles and inhomogeneities of temperature and humidity. Although most of these techniques have been developed for use at short range in the atmospheric boundary layer, microwave pulsed Doppler radar is one technique by which a ground-based installation can sometimes obtain wind measurements to quite high altitudes¹. Normally the spatial resolution of such radars is on the order of hundreds of metres. Here we report some observations of very strong shear made using a pulsed Doppler radar specially designed to achieve high spatial resolution.

The 25 m diameter aerial at Defford² has been coupled to a 10.7 cm wavelength radar (an FPS-18 on loan from the National Center for Atmospheric Research) redesigned to give a fine range resolution together with full Doppler measuring facilities. At present the peak power is limited to 320 kW. It has been modified to transmit frequency-modulated pulses of length 600 m. After reflection from targets these pulses are received, compressed and range-gated to give a resolution in range of 30 m. This is comparable with the beam-width resolution at a range of about 5 km.

The high gain of the aerial (53.2 dB), the low receiver noise temperature (170 K) and compression ratio (15 dB) produce a high sensitivity. The minimum detectable reflectivity for an extended target is $1.2 \times 10^{-15} \text{ m}^2 \text{ per m}^3$ at a range of 5 km. Two phase sensitive detector outputs from the receiver give the in phase and quadrature components of the Doppler frequency. They enable velocities to be measured unambiguously over the range $\pm 25 \text{ ms}^{-1}$. As large returns from the ground, received in the aerial side-lobes, tend to mask the wanted targets, a range gated MTI (moving target indicator) has been built to filter out the stationary targets. This device has 256 gates, each 30 m wide, covering a total range of 7.5 km which can be set anywhere between 0 and 100 km.

The methods of recording the data are shown in Fig. 1. The output of the MTI is displayed on a rapidly processed photo-

Fig. 2 Vertical profiles of wind shear and Richardson number derived over layers 430 m deep from the radiosonde released at 1450 GMT.



graphic strip chart to provide an intensity modulated time-range record of target reflectivity in real time. Doppler information from 30 selected range gates covering a total range of 900 m are also recorded on audio tape. This can be replayed rapidly and frequency spectrum analysed to give an RV (range-velocity) display on a colour television. Different colours represent different levels of echo intensity in each element of the RV matrix. If other batches of 30 range gates need to be processed similarly they are extracted from a video recording of the raw radar data.

Radar observations described here were obtained between 1446 and 1454 GMT on 30 March 1977. A balloon-borne radiosonde released during this period from the radar site showed a strongly sheared layer of strong static stability in the middle troposphere between 6.0 and 7.7 km. The Richardson number over much of this layer, evaluated over 430 m height intervals (Fig. 2), was close to 0.25. This is regarded as the critical value for the onset of Kelvin Helmholtz (KH) shearing instability³.

The radar beam was fixed throughout the period of the observations, looking at an elevation angle of 60° into the direction (330°) of both the wind and the wind shear vector over the strongly sheared layer. The 60° elevation angle was a compromise which enabled the radar to detect a major component of the horizontal wind at the same time as obtaining a nearly vertical profile of this component. Figure 3a is a print of the rapid processor time-range display. The ordinate is labelled in terms of true height allowing for the 60° elevation of the radar beam. Figure 3a shows the pattern of echo received mainly from small ice particles at the top of a cirrus cloud deck. Before 1440 the cloud top consisted of gently and uniformly inclined streamers of ice particles falling at less than 1 ms^{-1} . Figure 3a shows that, for a time after 1440, these streamers were distorted in a wave-like manner in the strongly sheared layer between 6.0 and 7.7 km. The crests of the waves were displaced to the right in the upper half of the layer and the troughs were displaced to the left in the lower half of the layer so as to be situated almost directly beneath the crests in the upper part of the layer. This is consistent with the pattern of air motion associated with large-amplitude KH billows⁴. This paper illustrates the capability of the radar to reveal the regions of strong wind shear which were associated with these billows.

Two examples of the format of the basic high-resolution range-velocity data are shown in Fig. 4. Velocity-height matrices such as these were obtained at 3 s intervals. Each element in the matrix is 26 m in height (30 m in slant range) by 0.625 ms^{-1} in radial velocity. As the likely fall speed of the ice particle tracers and the maximum vertical air velocity inferred from the slope of the streamlines of Fig. 3a was small ($< 1 \text{ ms}^{-1}$) compared with the horizontal wind ($> 20 \text{ ms}^{-1}$), the horizontal wind velocity can, to a first approximation, be taken as twice the indicated radial Doppler velocity component. Using such data, we have derived a time-height diagram of the inferred horizontal wind speed for the top half of the disturbance (Fig. 3b). We have also derived the pattern of vertical wind shear over 52 m height intervals (Fig. 3c). Although neglect of vertical air motions does create errors in wind shear of up to $\pm 30\%$ in places, a corrected pattern of wind shear which has been derived, whilst different from Fig. 3c in detail, does not dramatically alter the overall pattern of shear.

At 1436 GMT, before the billows developed, the Doppler measurements had shown that the shear over the 400 m height interval between 6.75 and 7.15 km was uniform at $2.5 \times 10^{-2} \text{ s}^{-1}$. At times, however, during the period depicted in Fig. 3 the shear within this height interval increased locally by as much as a factor of 8, when evaluated over 52 m height intervals. The resulting shear, $20 \times 10^{-2} \text{ s}^{-1}$, can be regarded as very strong for the free atmosphere. The small vertical extent and the transient nature of the regions of strong shear would make them difficult to observe by more conventional *in situ* sensing techniques.

A pulsed microwave radar technique for obtaining high resolution measurements of wind shear has been described. The

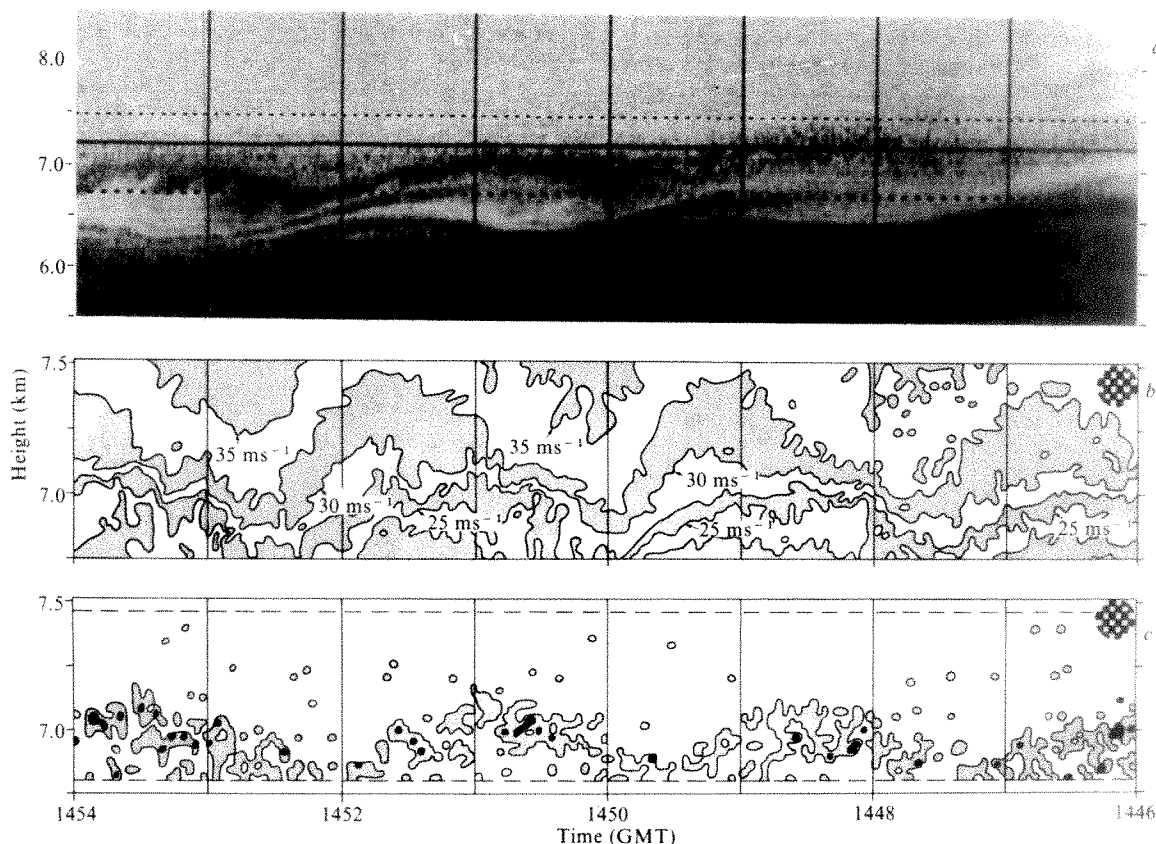


Fig. 3 Time-height record of (a) intensity of radar echo between 1446 and 1454 GMT on 30 March 1977 showing wave-like perturbations at the top of a layer of cirrus cloud. Assuming that the pattern was advecting at the speed of the wind at the middle of the perturbed layer, the wavelength would have been 3.4 km. Time-height records are also shown of (b), inferred horizontal wind velocity and (c), inferred vertical wind shear computed over layers 52 m deep. The data in (b) and (c) are for the height interval shown between dotted lines in (a): note the change in vertical scale. Isopleths of velocity in (b) are at 2.5 ms^{-1} intervals. In (c) regions of vertical wind shear exceeding $5 \times 10^{-2} \text{ s}^{-1}$ and $10 \times 10^{-2} \text{ s}^{-1}$ are dotted and black, respectively.

technique has been illustrated by means of a case study in which a vertical wind shear as strong as 0.20 s^{-1} was measured at an altitude of 7 km. The case described utilised small ice particles as tracers of the air motion. It would also work using signals back-scattered from refractive index inhomogeneities associated with humidity and/or temperature fluctuations which, with the present radar, are detectable most of the time in the boundary layer and for some of the time in the upper atmosphere, especially where there is strong shear and turbulence. As the radar operates at a wavelength virtually unattenuated by rain and water vapour it has an all-weather capability. High spatial resolution and an all-weather capability can also be achieved using frequency modulated continuous wave (FMCW) Doppler

radar techniques⁵ but so far such techniques have been restricted mainly to measuring winds in the atmospheric boundary layer.

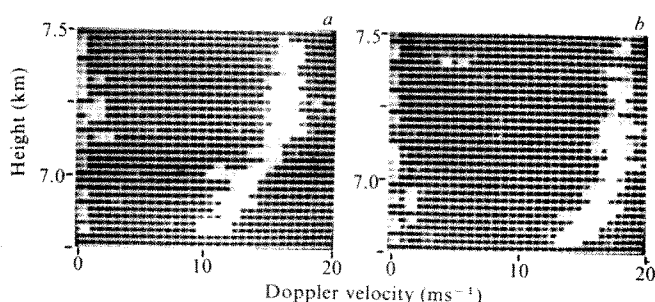
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Fig. 4 Monochrome photographs of the Doppler velocity versus height colour TV display at (a), 1449.00 and (b), 1449.57 GMT showing the region of wind shear and its change in height between the crest and trough of a billow (colours in the original display represented the intensity of the radar echo).



Distribution of helium in metal tritides

THE first generation of fusion reactors will utilise the reaction between deuterium and tritium nuclei. Various metal tritides, therefore, have potential applications for the transport, storage, purification, isotope enrichment and gettering of tritium. Although many properties of the metal tritides can be estimated¹ from the corresponding hydrides and deuterides, the effects of the radioactive triton decay into a β -particle and ^3He atom with a half life of 12.3 yr can only be determined by direct observation. Considering the expected² insolubility of inert ^3He atoms in metal lattices, it is surprising that many

metal tritides^{1,3,4} contain ³He/T atom ratios in excess of 0.10. The physical state of the retained ³He in these tritides is a very interesting and unresolved problem. Weaver and Camp^{5,6} suggest the ³He atoms are trapped in the octahedral interstitial sites for tritides with fluorite crystal structures while Bowman, *et al.*¹ believe the ³He atoms primarily reside in microscopic gas bubbles with dimensions < 1,000 Å. Here we describe nuclear magnetic resonance (NMR) studies of the relaxation times for ³He nuclei in LiT, TiT_{1.9}, and UT₃, which are representative of ionic, transition metal, and actinide tritides. These NMR measurements support the ³He bubble concept and further indicate an increase in the mean bubble dimensions as the samples age.

The NMR relaxation times are directly related⁷ to the physical state of the resonant spins. When the nuclei are immobile on lattice sites in a solid, the relaxation times obey the relations

$$T_2^* \approx T_2 \ll T_1 \quad (1)$$

where T_2^* is the linewidth relaxation time which corresponds to the experimental linewidth, T_2 is the homogeneous spin-spin relaxation time, and T_1 is the spin-lattice relaxation time. $T_2 < 50 \mu\text{s}$ are typically observed⁷ for rigid atoms in a solid. The molecular motion in macroscopic quantities of bulk gas yields

$$T_2^* < T_2 \approx T_1 \quad (2)$$

The differences in the relaxation times for rigid and mobile spins are dramatic with T_2^* and T_2 increasing by 2–4 orders of magnitude. The measured ³He relaxation times in LiT, TiT_{1.9}, and UT₃ are summarised in Table 1. Standard pulse NMR techniques⁸ were used to determine these relaxation times. The tritides were prepared by reactions at elevated temperatures between pure metals and tritium gas. Samples were sealed in evacuated glass tubes before commencing the NMR studies. The nonexponential decays generally observed during the NMR measurements are indicative^{9,10} of a variety of localised and isolated environments for the ³He spins (that is clusters or microbubbles).

Although the absolute magnitudes of the ³He relaxation times are different for LiT, TiT_{1.9}, and UT₃, these tritides have a common obedience to

$$T_2^* < T_2 < T_1 \quad (3)$$

rather than equation (1) or (2). Furthermore, T_2 and T_1 for ³He in each tritide become significantly longer with age (as the retained ³He contents increase). This behaviour implies a remarkable similarity in the ³He distributions for materials with very different¹ physical characteristics. The observed T_2^*

reflect the relative inhomogeneous line broadening contributions arising from the bulk magnetic susceptibilities¹¹, which accounts¹⁰ for the very short T_2^* in the strongly paramagnetic UT₃.

Nuclear relaxation times for ³He atoms in gas bubbles can be represented by the phenomenological expressions

$$T_1^{-1} = T_{1B}^{-1} + T_{1W}^{-1} \quad (4)$$

$$T_2^{-1} = T_{2B}^{-1} + (\lambda/R)T_{2W}^{-1} \quad (5)$$

where T_{1B} and T_{2B} correspond to bulk gas, T_{1W} is the spin-lattice relaxation at the bubble wall, R is the average bubble radius, and T_{2W} is the spin-spin relaxation time in a surface film of depth λ (approximately 2–3 atom layers). As spin relaxation in bulk monoatomic ³He gas is very inefficient⁷, $T_{2B} \approx T_{1B} > 10^3\text{s}$, and equations (4) and (5) are dominated by the wall relaxation processes. Although the surface relaxation mechanisms are not fully understood, any surface paramagnetic species will rapidly relax ³He spins during collisions with the bubble wall. Assuming relaxation occurs only in the short ($\sim 10^{-13}\text{s}$) collision sticking time t_s , T_{1W} is predicted¹⁰ to be

$$T_{1W} = (4R/3 < v > t_s) T_{1S} \quad (6)$$

where $< v >$ is the average thermal velocity of ³He atoms and T_{1S} is the surface relaxation time. As T_{1S} should be extremely sensitive to the electronic structure and chemical composition of the host material, very different ³He T_1 values for each tritide are expected and observed in Table 1. Furthermore, equation (6) predicts T_1 becomes longer as the bubble radius R is increased. T_{2W} can also be described by a relation similar to equation (6) where $T_{2S} < T_{1S}$ because the semi-static dipolar interactions which dominate T_2 relaxation⁷ would be strongest between ³He atoms near the wall. Consequently, ³He spin relaxation in bubbles would obey $T_2 < T_1$, and T_2 , with an approximately R^2 dependence, would increase more rapidly than T_1 as the bubbles enlarge.

The ³He relaxation times in Table I for LiT, TiT_{1.9}, and UT₃ are in good qualitative agreement with growing microscopic bubbles for the helium distributions in these tritides. Unfortunately, several factors prohibit a more definitive analysis. First, a range of bubble sizes (each with its own characteristic T_1 and T_2) as described by Gruber¹² is more likely than uniform bubble dimensions and the NMR studies will correspond to an 'average' bubble dimension. Various magnetic field gradients also contribute¹⁰ to the observed ³He relaxation times and tend to magnify the R -dependence for T_2 . The T_{1S} and T_{2S} values are unknown for these materials and may also have inherent time dependences which could modify the relationships between observed ³He relaxation times and bubble dimensions. Nevertheless, the ³He relaxation times observed in LiT, TiT_{1.9}, and UT₃ have been shown to be more consistent with helium atoms in microscopic bubbles whose average dimensions increase with age than the alternative explanation^{5,6} of rigidly trapped interstitials. The absence of known T_{1S} and T_{2S} values precludes a reliable determination of the bubbles dimensions from the NMR data in Table 1. However, bubble radii in the range of 10–100 Å are reasonable estimates. High resolution electron microscopy studies are required to establish better the bubble size distribution in the metal tritides.

I thank Dr A. Attalla for help with the NMR experiments.

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Table 1 Measured ³He relaxation times in LiT, TiT_{1.9} and UT₃. The NMR resonance frequency was 20 MHz for UT₃ and 45.7 MHz for LiT and TiT_{1.9}.

Material	Age (d)	T_2^* (ms)	T_2 (ms)	T_1 (ms)
LiT	184	0.23	15	6,500
LiT	346	0.47	26	7,800
LiT	541	0.44	35	9,350
LiT	735	1.04	125	10,000
TiT _{1.9}	168	0.049	0.9	500
TiT _{1.9}	340	0.062	3.2	810
TiT _{1.9}	671	0.065	10.2	1,200
TiT _{1.9}	933	0.072	45	1,500
UT ₃	150	0.020	1.1	35
UT ₃	366	—	6.1	39.5
UT ₃	836	0.020	16.0	74
UT ₃	1108	0.024	32.3	93

The T_2 values were obtained using the Carr–Purcell–Meiboom–Gill pulse sequence⁸ with a 200 μs spacing between the 180° pulses. All measurements were performed at room temperature.

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Exsolution in almandine-pyrope-grossular garnet

MINERALS of the garnet group are common in metamorphic rocks and in xenoliths derived from the Earth's mantle. They consist of multicomponent solid solutions with the general formula $X_3^{2+}Y_3^{3+}Si_3O_{12}$, where X represents Fe^{2+} , Mg^{2+} , Mn^{2+} , Ca^{2+} in 8-coordination, and Y represents Al^{3+} , Fe^{3+} , Cr^{3+} in 6-coordination. Solid solution between $Fe_3Al_2Si_3O_{12}$ (almandine), $Mg_3Al_2Si_3O_{12}$ (pyrope) and $Ca_3Al_2Si_3O_{12}$ (grossular) had been generally thought to be complete at temperatures appropriate to metamorphism. This paper reports the observation of exsolution in a natural garnet of this series from a high-grade metamorphic rock. Electron microscopic techniques have been used to demonstrate the phase separation of iron-rich precipitates from a more calcic matrix.

Theoretical considerations of the approximate thermodynamic properties of pyrope-grossular solid solutions by Ganguly and Kennedy¹ suggested the possibility of exsolution below about 700 °C, but attempts to demonstrate this experimentally proved inconclusive. Experimental data on the mixing properties of almandine-grossular solid solutions indicate the probable existence of a miscibility gap in this system at temperatures as high as 900 °C (ref. 2). The two-phase region calculated from these data occurs towards almandine-rich bulk compositions. These mixing data will be published elsewhere³.

In order to verify the experimental results a natural garnet of bulk composition almandine_{0.49} pyrope_{0.21} spessartine_{0.01} grossular_{0.29}, described by Wood⁴ from a metagabbro (specimen 16) in South Harris, was investigated by scanning and transmission electron microscopy. The metagabbro has been metamorphosed in granulite facies conditions, and Wood⁴ has determined the probable equilibrium pressure and temperature at the peak of metamorphism to be 10–13 kbar and 800–860 °C. The bulk composition of the garnet from this rock lies within the experimentally indicated two-phase field. Scanning electron micrographs of this garnet etched with 5% HF solution show preferential etching which produces surfaces composed of roughly spherical-shaped particles of various sizes up to about 0.5 µm diameter. The garnet seems to be composed of a microaggregate of particles.

Transmission electron micrographs of ion-thinned samples show the garnet to be composed of many individual polyhedral particles, set in a matrix which has been more easily etched by the ion-thinning process (Fig. 1). The size of individual polyhedra is variable up to about 0.4 µm, but most are less than 0.1 µm in diameter. The larger polyhedra often appear to be distributed in zones within the garnet (Fig. 1b). Contrast features in the matrix also suggest a finer scale of precipitation. The size and distribution of exsolved polyhedra may reflect the retrograde pressure temperature history of the rock, but further coarsening of exsolution textures at low temperatures may be hindered by small diffusion coefficients for garnet.

Analytical electron microscopy at 100 kV using EMMA-4 (ref. 5) has enabled the chemistry of the polyhedra and of the matrix to be determined. By focusing the beam into a small probe it is possible to analyse individual areas of 0.1–0.2 µm diameter. The matrix and polyhedra both have garnet stoichiometry, but the matrix is richer in calcium than the adjacent polyhedra. Results of chemical analyses calculated as mole fractions of grossular component (Ca/Ca + Mg + Fe) are shown

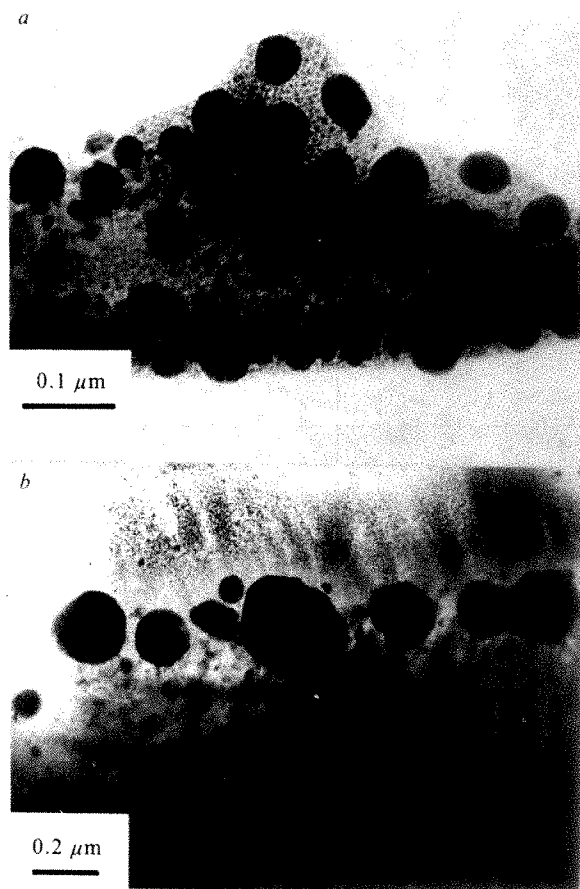


Fig. 1 Transmission electron micrographs of ion-thinned garnet, showing exsolved iron-rich garnet polyhedra. The polyhedra appear dark, partly due to diffraction contrast and partly due to thickness, as the more calcium-rich garnet matrix is preferentially etched during the ion-thinning process. Smaller precipitates are also visible in the matrix. *a*, Scale bar 0.1 µm; *b*, 0.2 µm.

in Table 1. Defocusing the electron beam to cover an area containing both exsolved polyhedra and matrix gives an analysis representative of the bulk composition, which is in good agreement with conventional microprobe results⁴.

An electron diffraction pattern from an area including many small polyhedra within the matrix is shown in Fig. 2. Dark field microscopy indicates that the regular array is from the matrix and the spotty powder rings from the randomly orientated polyhedra; both index as garnet. Cell-edges calculated from the regular array and from the powder rings differ relatively by 0.12 Å, with the regular array from the garnet of larger cell-edge. This value of 0.12 Å corresponds to a compositional difference in pyrope-almandine-grossular solid solutions of about 0.25 mole fraction of grossular component, in agreement with the chemical difference found using EMMA-4 and with the

Table 1 Garnet analyses

	Polyhedra	EMMA-4 Matrix	Bulk	Microprobe ⁴ Bulk
Ca/Ca + Mg + Fe	0.22	0.37	0.28	0.29
(mol fraction)	0.20	0.32	0.29	
	0.19	0.40	0.29	
	0.21	0.35	0.26	
Average	0.20	0.36	0.28	0.29

EMMA-4 analyses represent a minimum compositional separation, as with a probe size of 0.1–0.2 µm it is often not possible to analyse single polyhedra without including some matrix. Also, the matrix often contains very small precipitates. Therefore, areas suitable for analysis of mainly single phases were carefully chosen.

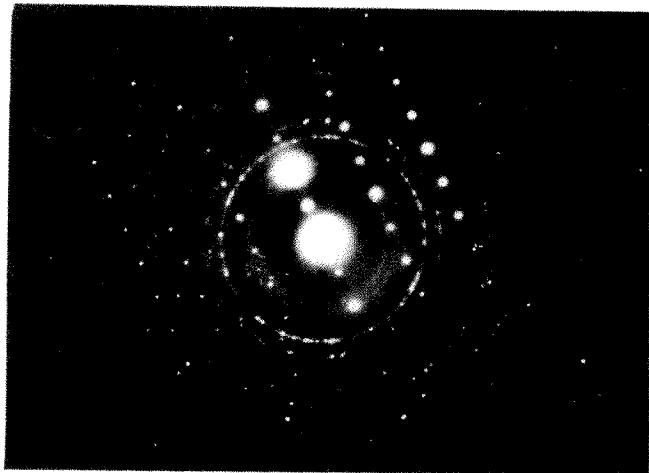


Fig. 2 An electron diffraction pattern from an area including many small polyhedra within the matrix. The regular array is from the matrix and the spotty powder rings from the randomly orientated polyhedra. Garnet cell-edges differ by 0.12 Å.

experimental mixing data. The diffraction pattern is, therefore, consistent with the interpretation that randomly-orientated garnet polyhedra have exsolved from a more calcium-rich garnet matrix.

Two distinct garnet phases produced by an exsolution mechanism are likely to be observed in almandine-pyrope-grossular garnets from metabasic rocks, as garnets from this paragenesis generally have more calcic bulk compositions (up to 30 mol % grossular) than those from metapelites. Exsolution may also occur in more complex garnet solid solutions which contain the components $\text{Ca}_3\text{Cr}_2\text{Si}_3\text{O}_{12}$ (uvarovite) and $\text{Ca}_3\text{Fe}_2\text{Si}_3\text{O}_{12}$ (andradite) in addition to almandine, pyrope and grossular end-member components.

I thank Drs B. J. Wood, R. Schmid, P. E. Champness and G. W. Lorimer for helpful discussions. The research was carried out during the tenure of a NERC research studentship.

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Received 14 September; accepted 14 December 1977.

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Standards for stable isotope measurements in natural compounds

RESEARCH based on stable isotope variations in natural compounds is expanding in scientific fields such as geochemistry, hydrology, environmental studies and biochemistry. However, intercomparison of results obtained in different laboratories is often not fully reliable and therefore to improve the intercalibration of deuterium and ^{18}O measurements in natural waters, two water standards have been distributed by the International Atomic Energy Agency since 1968. The two standards, called V-SMOW (Vienna Standard Mean Ocean Water) and SLAP (Standard Light Antarctic Precipitation), were prepared, following a recommendation by an Advisory Group Meeting convened by the IAEA in 1966. Information on the two standards is given here.

V-SMOW was obtained by mixing distilled ocean water with small amounts of other waters in order to bring its isotopic composition as close as possible to that of the defined SMOW¹.

According to the control analyses made by Craig, V-SMOW has the same $^{18}\text{O}/^{16}\text{O}$ ratio as the defined SMOW, but a slightly lower D/H ratio (0.2‰). This difference in deuterium content, however, is 5 to 10 times lower than the experimental error of many laboratories.

The D/H absolute ratio² of V-SMOW was $(155.76 \pm 0.05) \times 10^{-6}$. The $^{18}\text{O}/^{16}\text{O}$ absolute ratio³ was $(2005.20 \pm 0.45) \times 10^{-6}$. No determinations were made of the $^{17}\text{O}/^{16}\text{O}$ ratio. The absolute maximum density of V-SMOW is 999.975 kgm⁻³ (see ref. 4). The tritium content of V-SMOW, determined at the IAEA by direct gas counting, was 18.5 ± 3.6 T.U. on 16 September 1976 (1 T.U. (tritium unit) corresponds to 1 atom of tritium per 10^{18} atoms of hydrogen).

SLAP was obtained by melting a sample of firn collected at Plateau Station, Antarctica. Its D/H absolute ratio² was $(89.02 \pm 0.05) \times 10^{-6}$. The tritium content of SLAP was 374 ± 9 T.U. on 16 September 1976.

Two other water standards, the so-called NBS-1 and NBS-1A—formerly kept at the National Bureau of Standards, Washington DC, USA, and now at the IAEA in Vienna—were also distributed together with V-SMOW and SLAP.

In September 1976, a consultants' meeting was convened by IAEA to review the results obtained by different laboratories for the above water standards, to discuss the intercalibration

Table 1 Deuterium intercalibration results expressed in $\delta\text{D}_{\text{‰}}$ against Vienna-SMOW

Laboratory*	NBS-1	NBS-1A	SLAP
1	-45.1	-180.3	-424.2
6	-47.8	-179.6	-420.4
9	-46.4	-181.0	-423.2
11	-47.6	-182.2	-426.5
12	-47.5	-182.7	-428.5
14	-49.2	-182.9	-435.0
16	-47.2		
17	-46.3	-181.2	-424.0
18	-45.7	-182.1	-425.4
20	-47.2	-183.1	-426.1
23	-45.4	-181.2	-430.6
24	-48.5	-183.2	-427.9
26	-46.6	-180.4	-418.1
27	-44.7	-179.6	-420.2
28	-48.6	-182.0	-424.2
29	-45.1	-181.0	-421.8
30	-45.8	-183.7	-428.3
31	-46.9	-182.4	-242.4
32	-46.9	-185.7	-436.2
35	-46.1	-181.1	-423.5
36	-47.4	-181.4	†
37	‡	‡	-428.7
38	-48.0	-183.5	-428.2
39	-49.8	-194.2	-452.2
40	-46.5	-182.0	-425.8
41	-49.0	-182.6	-428.5
44	-48.8	-182.0	-425.5
45	-46.0	-184.0	-428.3

*Nos refer to laboratory identification included in reprints.

†Not measured, but measurements made on individual batches of Antarctic water before mixing to produce the SLAP ranged from -423 to -434 (Craig, personal communication), with an average value of -429.2 against Vienna-SMOW.

‡The SLAP value has been obtained by assuming for NBS-1 and NBS-1A the δD values of -47.6 and -183.3‰ corresponding to those reported by Craig against the defined SMOW.

of stable isotope measurements in natural samples and to receive advice on future activities. A short summary of the discussions and of the recommendations made by the consultants is given below. A more detailed report can be obtained from IAEA.

Sets of V-SMOW, SLAP, NBS-1 and NBS-1A have been distributed to 114 laboratories representing 30 countries and 2

Table 2 ¹⁸O intercalibration results in δ¹⁸O‰ against Vienna-SMOW

Laboratory	NBS-1	NBS-1A	SLAP
1	-7.62	-24.38	-54.92
2	-7.67	-23.99	-55.19
3	-7.72	-24.09	-55.35
4	-7.81	-24.16	-55.46
5	-8.01	-24.40	-56.07
7	-8.12	-24.37	-55.73
8	-7.90	-24.43	-53.92
9	-8.01	-24.52	-56.38
10	-7.85	-23.97	-55.04
11	-7.9	-24.65	-56.5
12	-7.98	-24.59	-56.50
13	-7.88	-24.28	-55.68
14	-7.97	-24.94	-56.46
15	-7.74		-55.0
16		-24.16	
17		-24.31	
18	-7.92	-24.36	-55.40
19	-7.77	-24.17	-55.17
20	-7.93	-24.18	-54.84
21	-8.09	-24.63	-56.09
22	-7.3	-23.45	-54.9
24	-7.76	-24.36	-55.02
25	-7.83	-23.90	-54.74
26	-7.94	-24.40	-55.49
27	-7.96	-24.62	-56.14
28	-7.90	-24.74	-56.32
29	-7.86	-24.28	-55.48
30	-7.89	-24.44	-55.75
31	-7.77	-24.08	-55.05
32	-7.86	-24.32	-55.57
33	-7.87	-24.28	-55.44
34	-8.04	-23.80	-55.75
36		-24.27	
37	-7.90	-24.26	-55.44
39	-7.86	-24.22	-55.20
40	-8.01	-24.38	
42	-7.92	-24.16	-54.53
43	-7.6	-24.5	-49.2
44	-8.05	-23.87	-54.91
45	-7.92	-24.10	-55.25

international organisations. The results submitted up to September 1976 are listed in Tables 1 and 2. The agreement among the different laboratories is reasonably good, and improves significantly by eliminating the results deviating from the average of more than two standard deviations (Table 3).

If fixed δ-values are assigned to SLAP and the δ values of NBS-1 and NBS-1A are normalised accordingly, the agreement of the results should improve. In fact, the measurement technique based on two standards should produce, in principle, results which compare better between different laboratories. In this case, however, the improvement is significant only for the δD value of NBS-1A, as shown by the decrease of the standard deviation.

To resolve confusion due to the expression of results from different laboratories in non-corresponding scales, it is recommended that all future results be expressed as δ-values relative to V-SMOW. Coherence between δ-values reported by different laboratories can be improved by adopting common δ-values for a second water reference standard with respect to which the ¹⁸O and deuterium δ-scales should be normalised. It is recommended, for this purpose, to adopt the water SLAP distributed by the Agency. The recommended δ-values for SLAP relative to V-SMOW are:

δ ¹⁸O (SLAP) = -55.5‰

δD (SLAP) = -428‰

These δ-values for SLAP were selected using data in Tables 1 and 2.

The adoption of V-SMOW as zero of the δ-scales and of prefixed values for SLAP corresponds in principle to a modification of the δ-scale which becomes:

δ = (R_{sample} - R_{V-SMOW}) / R_{V-SMOW} δ_{SLAP} × [(R_{SLAP} - R_{V-SMOW}) / R_{V-SMOW}] (1)

where R = ¹⁸O/¹⁶O or D/H and δ_{SLAP} is the value adopted for ¹⁸O or deuterium content of SLAP with respect to V-SMOW. The classical definition of δ as the relative difference (generally reported in ‰) of the isotopic ratio with respect to SMOW is:

δ = (R_{sample} - R_{SMOW}) / R_{SMOW} (2)

The two δ-scales coincide if R_{SMOW} = R_{V-SMOW} and if the adopted δ-values for SLAP correspond to the true ones as defined by equation (2). These two conditions seem to be fairly well fulfilled and therefore in practice equations (1) and (2) coincide. In addition, errors of δ which can be introduced by an incorrect assessment of multiplicative correction factors automatically cancel from equation (1).

Therefore, the advantages of significantly improving the correct comparison of results obtained in different laboratories by the adoption of recommended δ-values for SLAP far outweigh the consequences of the implications discussed above.

The need for a third reference water sample with isotopic composition approximately midway between V-SMOW and SLAP was also recognised. This sample has been called GISP (Greenland Ice Sheet Precipitation). The IAEA will soon start distributing it, with the aim of calibration on the V-SMOW/SLAP scale.

It was agreed that most of the stock of V-SMOW, SLAP and GISP should be stored in sealed flasks of approximately 10 l each. Some of the flasks should be kept at the US National Bureau of Standards, in Washington, and the rest at the IAEA in Vienna.

About 10 l of each standard should be stored in sealed pyrex vials of about 25 ml for distribution by IAEA. In principle, V-SMOW, SLAP and GISP should be distributed upon request only, but laboratories are encouraged to make a precise calibration of their working standards on the V-SMOW/SLAP scale about once every three years.

The establishment of other stable isotope standards is required in order to improve the intercomparison of results obtained in different laboratories in natural substances other

Table 3 Intercalibration average values and standard deviations

Sample	Deuterium			¹⁸ O		
	δ	σ	n	δ	σ	n
NBS-1	1. -47.0	1.4	27	-7.87	0.16	37
	2. -47.0	1.4	27	-7.88	0.13	36
	3. -47.1	1.2	26	-7.90	0.18	36
	4. -47.1	1.2	26	-7.89	0.12	34
NBS-1A	1. -182.6	2.8	26	-24.28	0.28	39
	2. -182.2	1.5	25	-24.29	0.23	37
	3. -182.9	1.1	26	-24.39	0.62	35
	4. -183.2	0.7	24	-24.29	0.25	34
SLAP	1. -427.1	6.6	26	-55.27	1.20	36
	2. -426.1	4.2	25	-55.45	0.16	35

- 1. All results included as reported in Tables 1 and 2.
- 2. Excluding those deviating by more than 2σ from the average of 1.
- 3. Results normalised with respect to SLAP, assuming for the latter δD = -428 and δ¹⁸O = -55.5. All results included.
- 4. Excluding the results deviating by more than 2σ from the average of 3.

Table 4 Proposed new stable isotope standards

Compound	Elements for which standard can be used	Remarks (and workers willing to investigate the possibility of procuring standards)
CaCO ₃	C, O	Calcite as close as possible to PBD. I. Friedman (Denver, Colorado) and J. O'Neil (Menlo Park, California).
CaCO ₃	C, O	Carbonatite. H. Friedrichsen (Tübingen, FRG).
CO ₂	C, O	Two different gas samples for mass spectrometer calibration. T. B. Coplen (Reston, Virginia).
C ₁₇ H ₁₁ O ₆	H, C, O	Tristearin for studies of organic compounds.
K ₂ Al ₄ (Si ₆ Al ₂ O ₂₀)(OH, F) ₄	H, O	Muscovite. I. Friedman and J. O'Neil.
K ₂ (Mg, Fe) ₆ (Si ₆ Al ₂ O ₂₀)(OH, F) ₄	H, O	Biotite. I. Friedman and J. O'Neil.
BaSO ₄	O, S	Obtained from sea water. Y. Horibe (Tokyo, Japan).
PbS or ZnS	S	Natural galena or sphalerite with $\delta^{34}\text{S}$ in the range -20 to -30‰. I. Friedman and J. O'Neil.
S	S	Sulphur derived from natural gas, depleted in ^{34}S . E. Roth (Saclay, France).
SO ₂	S	Two different gas samples for mass spectrometer calibration. J. O'Neil and I. L. Barnes (Washington D.C.).
(NH ₄) ₂ SO ₄	N	Two samples differing by about 20‰. E. Salati (Piracicaba, São Paulo, Brazil).
KNO ₃	N	I. Friedman and J. O'Neil.

The so-called PBD standard consisted of a CaCO₃ obtained from a belemnite of Peedee formation of South Carolina. Its ^{13}C and ^{18}O contents are supposed to be close to the average contents of marine limestone. The PDB standard was first adopted in the early 1950s as reference in palaeotemperature studies.

than water. The proposed new reference samples, which will be kept at both IAEA and US National Bureau of Standards, are listed in Table 4. They will supplement the standards now being distributed by the US National Bureau of Standards which, in some cases, are insufficient in amount for a long-term worldwide distribution (as, for instance, the samples of nitrogen (NBS-14) and of SO₂ (NBS-120A), I. L. Barnes, personal communication).

SLAP was prepared by H. Craig, University of California at San Diego; GISP was provided by W. Dansgaard, University of Copenhagen. A list of laboratories participating in the stable isotope intercalibration is contained in the reprints of this article.

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Received 29 June; accepted 1 December 1977.

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Silica oxygen isotopes in diatoms: a 20,000 yr record in deep-sea sediments

PALAEOTEMPERATURE determinations based on oxygen isotope composition of planktonic foraminifera are well established for Quaternary and Tertiary deep-sea sediments¹⁻⁴. Wide areas of the ocean floor, however, are lacking suitable calcareous fossils due to dissolution or unfavourable ecological conditions. Here we show that isotopic analyses of diatom frustules can adequately substitute for the analyses of calcareous microfossils. To test the efficiency of diatoms as an isotopic palaeothermometer^{5,6}, we compare the oxygen isotope records of diatoms and of planktonic foraminifera in a box-core from the eastern equatorial Pacific (PLDS 72, latitude 01°08'N; longitude 109°15'6''W; water depth 3,626 m). This core was among other box-cores raised during the S.I.O. Pleiades expedition between May and September 1976, in an effort to obtain undisturbed samples of the uppermost deposits in this fertile area of pelagic sedimentation.

Isotope analyses of the planktonic foraminifera followed

standard procedures⁷ and the reproducibility of the oxygen isotope values is about $\pm 0.1\%$. All box-cores were routinely assessed isotopically at Scripps Institution of Oceanography to establish stratigraphic continuity.

The preparation of biogenous silica for isotopic analysis requires considerably more effort than that of calcareous materials. The diatoms are separated from carbonate, radiolarians and detritals by HCl treatment, ultrasonication, sieving (fraction larger than 22 μm retained) and differential settling. The process further requires a complete cleaning of the silica which removes all extraneous materials as organic coatings and hydration water. A new method has been developed for this process which calls for 2 weeks of oxidation with 0.1M KMnO₄, 1 week of desiccation with P₂O₅ and partial recrystallisation under vacuum at 800–1,000 °C (ref. 8). Compared with an earlier cleaning method on recent diatoms and sponge spicules^{5,6}, a +7‰ isotopic shift was observed using the improved cleaning procedure on oceanic siliceous ooze. This shift is reproducible for replicate treatments. The lower δ values obtained by the first cleaning procedure are presumably due to residual organic carbon and hydration water reacting with the silica during the high temperature heating. Results to be published elsewhere⁹ show that although the magnitude of the fractionation factor between silica and water is changed by the more complete cleaning, this does not seem to affect the variations with palaeotemperature of the isotopic fractionation. Thus, while the scale for the absolute values of the silica $\delta^{18}\text{O}$ shifts as a function of preparation, the shape of the stratigraphic isotope signal would remain unchanged. The final cleaning and the isotopic analyses were made at Centre des Faibles Radioactivités, C.N.R.S., France. Reproducibility of the isotope values was 0.1‰.

Results show an overall parallelism between the oxygen isotope records of diatoms and of the planktonic foraminifera (Fig. 1a and b). The exact time span of the record is not known, but is immaterial for the present discussion. An age of 18,000 yr for the $\delta^{18}\text{O}$ maxima⁹ near a core depth of 34 cm yields an average sedimentation rate of 1.9 cm per 1,000 yr which seems reasonable for this area. Thus, the 40 cm long core represents about 20,000 yr. The oxygen isotopes show high values in the older sediments and low values in the younger ones, reflecting the familiar transition from glacial to Holocene time. The overall range from high to low $\delta^{18}\text{O}$ values is greater for the diatoms ($\sim 1.5\%$) than for the foraminifera ($\sim 1.2\%$). The transition may be uneven^{7,10} although the exact nature of this unevenness is obscured by mixing processes.

The oxygen isotopic ratio of planktonic foraminifera in deep-sea sediments is a function of the temperature and the

isotopic composition of the water in which they developed. Part of the development can take place within the thermocline, and the average depth of the shell precipitation is thus an important factor. The isotopic composition of seawater depends on the amount of continental ice^{1,3,12} and also on the evaporation/precipitation ratio, that is, regional salinity. The interpretation of oxygen isotope values is further complicated by preferential dissolution on the sea floor of isotopically lighter shells⁷. The magnitude of these various effects has been reviewed elsewhere¹¹. Analogous effects may be expected for diatoms, except that these photosynthesising, shallow-living algae preferentially develop their skeletons within the mixed layer of the ocean. Depth of growth may, therefore, be ruled out as an important factor. Seasonal changes of temperature and changes in the evaporation/precipitation ratio will affect diatoms as much as foraminifera. Direct input of melt water could conceivably produce a differential effect on diatoms in pericontinental and high latitude waters. Any such differential is thought to be insignificant in the area under study.

The deglaciation effect accounts for most of the foraminiferal isotopic range (Fig. 1b) being approximately 1.2‰ in magnitude^{3,12}. It should influence the diatoms in a similar way. Which other effects are then responsible for the measured 0.5‰ difference (Fig. 1c) in the isotopic range between the diatoms and foraminifera?

An effect of temperature is certainly possible, if the surface water inhabited by the diatoms experienced more warming than the deeper waters inhabited by the foraminifera *Neogloboquadrina dutertrei* and *Pulleniatina obliquiloculata*. *P. obliquiloculata* secretes its shell mostly in subsurface waters whereas *N. dutertrei* typically occurs in upwelling regions and hence reflects a strong influence of cold subsurface water in its composition. Of the other possible effects two deserve special attention: a change in species composition of the diatom assemblages (season effect) and changes in the preservation of the siliceous and calcareous microfossils (dissolution effect).

With the beginning of the post glacial, upwelling seems to have been reduced in the Equatorial Pacific¹³. The abundance of *Thalassionema nitzschioides*, a diatom species especially confined to areas of upwelling, is reduced at this point. The composition of the diatom assemblages otherwise remains virtually constant throughout the core interval and any seasonal influence on the isotopic values, therefore, seems negligible.

Differential dissolution of the less silicified warm-water frustules of diatoms can enrich an assemblage with cold-water forms¹⁴ and conceivably disturb the isotopic signal. In the present case, there is no evidence for interference from such dissolution effects. Dissolution has affected certain core levels but there is no great difference between diatom preservation in glacial and post-glacial sediments in this core (Fig. 1e).

There is evidence, however, that the foraminiferal signals have been affected by differential dissolution. In the present case, such dissolution would decrease the range of the foram signal, as it removes isotopically light shells from post-glacial sediments⁷. A noticeable divergence of the signals of *N. dutertrei* and *P. obliquiloculata* begins near 17 cm in the core. A similar divergence between two foraminifera species has been shown to mark the onset of the Holocene dissolution pulse 12,500 ¹⁴C years ago⁷. A decrease in per cent carbonate immediately follows (Fig. 1d). This level also corresponds to the divergence of the diatom and foraminiferal isotope signals (Fig. 1c).

It seems, therefore, that two effects are important in producing a difference in isotopic ranges between diatoms on one hand and foraminifera on the other: (1) a greater temperature sensitivity of diatoms (because they live in surface waters) and (2) a reduction in the foraminiferal isotopic range (due to the Holocene carbonate dissolution). If differential warming of surface and subsurface water could entirely account for the discrepancy between diatoms and foraminifera, surface waters would have warmed more by 1.5 °C, about one half of the total warming calculated by CLIMAP⁹ for this area. But the difference could also be a reflection of carbonate dissolution so that the

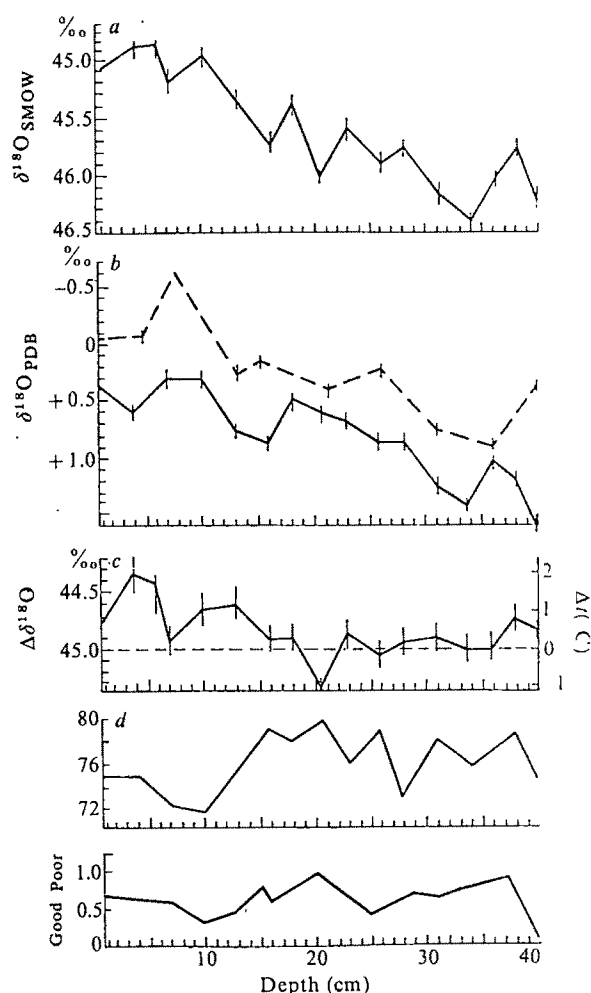


Fig. 1 Variation with depth in Core PLDS 72 Bx from the eastern Pacific of: a, $\delta^{18}\text{O}$ SiO_2 against SMOW of clean diatom valves after oxidation of organic matters with KMnO_4 , desiccation by P_2O_5 and high temperature heating (analyses by L. Labeyrie). b, $\delta^{18}\text{O}$ CaCO_3 against PDB of two foraminifera species: *Pulleniatina obliquiloculata* (dashed line) and *Neogloboquadrina dutertrei* (solid line) (analyses by J. S. Killingley and E. Vincent). c, Calculated $\Delta(\delta^{18}\text{O} \text{ SiO}_2 - \delta^{18}\text{O} \text{ CaCO}_3)$ (*N. dutertrei*). The reported Δt has been calculated from: $\Delta t \sim 4\Delta(\text{SiO}_2, \text{H}_2\text{O}) \sim 4\Delta(\text{CaCO}_3, \text{H}_2\text{O})$ (8) where zero is the mean value for the 18–40 cm depth range. d, Carbonate abundance, in per cent of weight. e, Diatom Preservation Index, a measure of the morphological change of the diatom valves caused by dissolution (analyses by N. Mikkelsen). In the relatively poorly preserved interval around 20 cm, dissolution resistant species like *Coccolithus nodulifer* and *Ethmodiscus rex* are concentrated¹⁶.

differential warming would be negligible.

In conclusion, the present study has shown the possibility of using the silica $^{18}\text{O}/^{16}\text{O}$ ratio in diatom valves as a palaeo-temperature indicator. The similarity between the diatom and foraminifera signals indicates that no isotopic re-equilibration between the diatom silica and the bottom water takes place. This is in contrast to findings by Mopper and Garlick¹⁵ who may have used inadequately cleaned samples. Comparison of $^{18}\text{O}/^{16}\text{O}$ ratios in diatoms and foraminifera from the same core may allow observation of changes in the thermal stratification of the oceans. The isotopic composition of diatoms will be a unique temperature tracer in high latitudes where shallow-dwelling foraminifera are commonly lacking in sediments. The isotopic composition of the diatom valves should also make it possible to extend the oxygen-isotope stratigraphy to deep-sea sediments below the compensation depth for calcium carbonate.

L. L. is supported by C.N.R.S., France and N. M. by the

Danish Natural SRC Grant 511-7182 and in part by Mobil Oil Corporation. The research further received support from the USNSF, Grant OCE-75-04335. We thank J. S. Killingley and E. Vincent for providing unpublished data and M. Kastner for helpful discussions.

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Received 1 November; accepted 5 December 1977.

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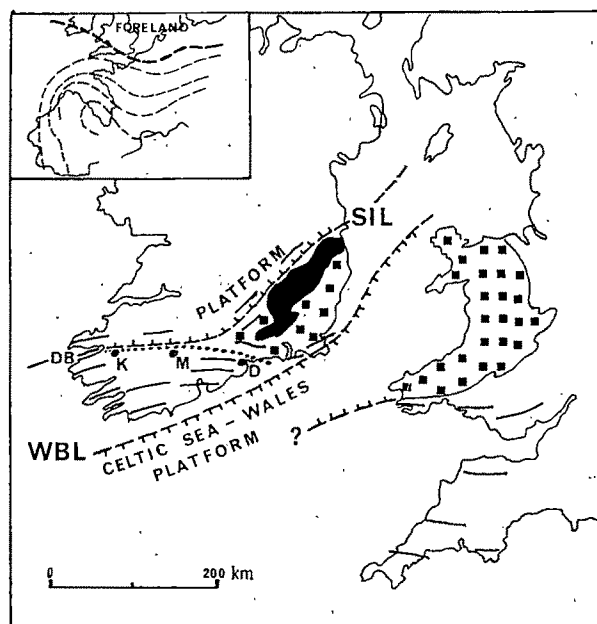


Fig. 1 Sketch map of Hercynian fold trends in Southern Ireland and south-west Great Britain. Blocked areas represent Lower Palaeozoic massifs, with the Leinster pluton and its concealed extension shown in solid. Dotted line indicates position of Hercynian Front in Ireland^{13,15}. SIL, South Ireland Lineament; WBL, Wexford Boundary Lineament; DB Dingle Bay; K, Killarney; M, Mallow; D, Dungarvan. Data sources from refs 14,25,26. Inset map showing location of Hercynian orogenic zones in Western Europe^{11,12} on a pre-Permian reconstruction²⁷.

Is the Hercynian Front in Ireland a local feature?

THE boundary of the northern foreland with the Hercynian orogeny in Europe—the Hercynian Front—has been recognised as a major feature in European palaeotectonic reconstructions^{1,2}, in transatlantic correlations³⁻⁷, and for metallogenic models⁸⁻¹². In all these assessments the Hercynian Front (or Thrust Front) is depicted as a structural line transecting southern Ireland on a crudely east-south-east (Armorican) trend across to South Wales. It is suggested here, however, that the 'Front' in Ireland has no direct link with South Wales, being separated by a major basement block, and may well be a localised internal foreland feature.

The position of the Hercynian Front in Ireland is based on the perceptive study by Gill¹³, who recognised a marked change in tectonic style across an arcuate line from Killarney to Dungarvan, part of which consisted of northerly directed thrusts. This he termed the Thrust Front, which was subsequently^{14,15} modified to continue westwards through Dingle Bay (Fig. 1). The acceptance of the line of the 'Front' here has led to recent attempts to trace it off the west coast of Ireland¹⁶ and to suggest a link with the Gibbs Fracture Zone^{5,6}.

An examination of the Hercynian fold trends in southern Ireland¹⁴, however, reveals three aspects at variance with the popular conception. First, south of the 'Front' the folds show an arcuate trace markedly divergent from the east-south-east Armorican trend except in the Dungarvan area; second, from Dungarvan eastwards the trend rotates back to an east-north-east direction (Fig. 2), negating the simple east-south-east extrapolation directly to South Wales; and third, the strike of Hercynian structures in the south of Ireland show close agreement with Lower Palaeozoic structural trends except to the north of Dungarvan, and hence can be regarded as having a Caledonoid trend rather than an Armorican one.

On this basis the concept of an Armorican trending Hercynian imprint on the south of Ireland, which transects

earlier Caledonoid trends and can be matched directly with that of south-west Wales and south-west England, cannot be upheld. Rather, the Hercynian structural pattern here is considered to have been locally basement controlled, and to reflect the influence of three major basement elements which resulted in contrasting 'platform' and 'basin' (graben) deformation¹⁷. These major elements are the South Ireland Lineament, the Wexford Boundary Lineament, and the Leinster pluton (Fig. 1). These two lineaments, which parallel Caledonoid structures, have been recently recognised as major temporally persistent palaeo features, influencing lower Palaeozoic deposition and deformation¹⁸ as block boundaries. Between, and controlled by, these two lineaments there was subsequently a thick Upper Palaeozoic accumulation (>4,000 m) in a structural basin¹⁹, thinning dramatically eastwards onto the positive block of the Caledonoid Leinster unit stiffened by its mid-Devonian pluton (Fig. 2).

Because of this eastward shallowing basement in the sector between the two lineaments, Hercynian compression here resulted in two differing structural provinces, a south-west 'graben' province, and a north-east 'platform' province. The former shows polyphase fold deformation^{13,20} with tight major fold structures and a locally penetrative cleavage fabric, together with severe northerly directed thrusting and structural dislocation in the Killarney-Dingle Bay area^{15,20} along the margin of the block delineated by the South Ireland lineament, and also further south at Kenmare²⁰ and Bantry (Gardiner, unpublished). Folds parallel the Caledonoid trend of this lineament, predictably swinging to an east-south-east orientation as deformation was influenced by the positive Leinster block¹³ in the Mallow-Dungarvan area. In contrast, in the Leinster platform area the Upper Palaeozoic cover shows only broad open folds due to the basement protection, and the folds are moulded against the Leinster pluton (Fig. 2). Post-Caledonoid flexing of the Lower Palaeozoics in south-west County Wexford²¹ and the inferred present day strike

swings of the two lineaments are also thought to be due to late stage Hercynian tightening against the rigid Leinster pluton.

In this structural model the northern boundary of the zone of 'graben' deformation therefore has an arcuate trace (Fig. 1) which is essentially the line of the recognised 'Thrust Front'¹¹⁻¹⁵. The major tectonic dislocations recorded at Mallow and further west^{13,15} seem, however, to be relatively localised features reflecting proximity to the edge of the northern positive block. Their apparently problematical absence east of Mallow is entirely predictable on this model, as this was a transitional zone between the 'graben' and 'platform' deformation areas, with deformation intensity decreasing northeastwards due to the progressive basement influence of the lower Palaeozoic Leinster block. This factor was recognised by Philcox²², who suggested that east of Mallow the 'Thrust Front' was split into a number of fanned 'arms' as it was accommodated by the Upper Palaeozoic cover above interfering basement units. Certainly there is no one continuous 'Thrust Front' in this area²³, as was earlier proposed¹³, and use of this term here would seem unsuitable.

Recognition of a positive basement block between south-east Ireland and South Wales during the Hercynian orogeny also creates problems in any simple linkage of the Hercynian Front between these two areas. Indeed, the Welsh Lower Palaeozoic massif seems to have also been part of this intervening structural platform, as the Hercynian fold trends in South Wales seem to have been influenced by the arcuate southern margin of the massif²⁵ (Fig. 1). The 'Thrust Front' position in south-west Wales is also a reflection of basement-cover interaction^{4,24}, although its position further southeastwards is by no means clear²⁵.

It is, therefore, concluded that there is no continuous linkage between the Hercynian Front in south-west Wales and Southern Ireland, in both areas its location and trend

being a function of basement control; simple metallogenic correlations are, therefore, improbable. Transatlantic correlation seems likely to relate to pre-Hercynian structural elements, and will depend on whether the western continuation of the 'Front' with the northern foreland is in fact along the South Ireland lineament or the southern margin of the Celtic Sea-Wales platform block (Fig. 1). If the latter, then the 'Thrust Front' in Ireland is perhaps a localised internal foreland feature. Certainly recent work off the west coast of Ireland¹⁶ indicates that the Gibbs Fracture zone is better related to the Great Glen Fault line rather than to the 'Hercynian Front' in Ireland. The inferred westerly closing of the other Hercynian orogenic zones in Western Europe when plotted on a pre-Permian reconstruction^{11,12} (Fig. 1, inset) would also accord with a continuation of the true Front southwestwards from south-west Wales. There is no evidence of proximity to a plate boundary directly off the south of Ireland, and the inferred evolutionary history of this area^{18,19} indicates that in Hercynian times there was no oceanic crust north of the Celtic Sea-Wales platform.

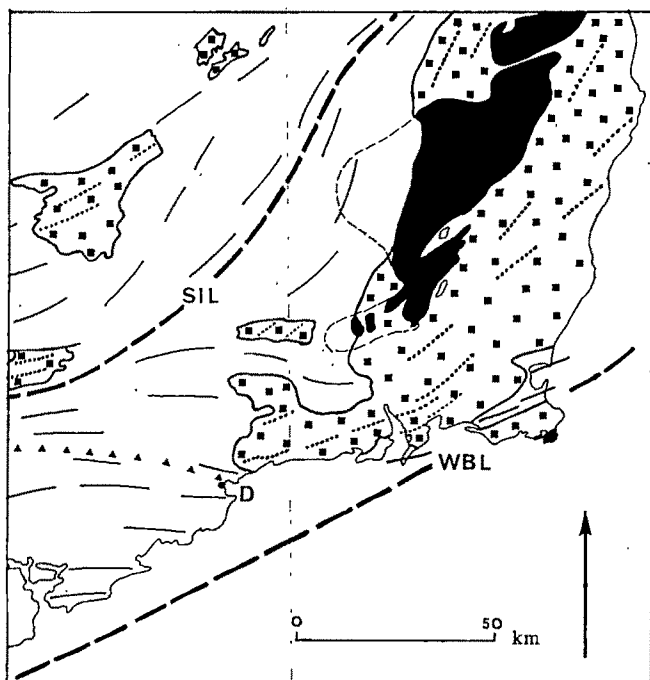
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Received 20 October; accepted 15 December 1977.

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Fig. 2 Sketch map of south-east Ireland, showing Caledonoid and Hercynian fold trends and the inferred positions of the South Ireland and Wexford Boundary Lineaments. Granites are in solid, with concealed extension of Leinster pluton shown by dashed line. Lower Palaeozoic and pre-Cambrian areas are blocked. ▲ show line of the Hercynian Front as recognised by Gill¹³. Other data sources from refs 14, 21, 26, 28.



Song repertoires and territory defence in the great tit

BIRD song is generally considered to have one or both of two main functions: attracting a mate and proclaiming a territory. The great tit (*Parus major*) is a typical songbird in that song is primarily a male vocalisation and it is produced before and during the breeding season (that is, from January to May in southern England). We have shown that great tit song plays a part in territory maintenance by experiments in which we 'occupied' territories with loudspeakers after removing resident pairs^{1,2}. New pairs seeking an empty space in which to settle moved into control areas more rapidly than into song-occupied territories. We report here on three experiments designed to study the effect of a varied song repertoire in territory maintenance. Each male great tit, in common with many other songbird species, has a repertoire of song variants some of which are usually shared between birds in a locality. In the great tit, repertoire size commonly varies between two and eight song types. In some species, for example the canary, elaborate repertoires seem to be favoured by sexual selec-

Table 1 Results from Marley Wood near Oxford showing that there is no evidence for female choice of males with large repertoires

Year		Repertoire size	
		1-3	4-6
1975	<i>N</i>	27	4
	Laying date*	31.9 \pm 0.62	35.6 \pm 3.5
	Clutch size*	7.7 \pm 0.21	7.3 \pm 0.5
	Per cent adult	59	50
1976	<i>N</i>	8	5
	Laying date	32.7 \pm 2.03	28.6 \pm 2.82
	Clutch size	8.1 \pm 0.44	8.2 \pm 0.58
	Per cent adult	75	60

This result is not surprising since females choose mates before the spring peak of male song. If females did prefer males with larger repertoire sizes, one would predict a correlation between repertoire size and breeding date or female fecundity. The age-corrected data show no such trend. The table also shows that males with larger repertoires do not tend to be older.

*Corrected for age of ♀

†Time in days after April 1.

tion^{3,4}, but we have found no evidence for this in the great tit (Table 1), in which pairing takes place before the spring peak of song. We therefore tested the possibility that repertoires enhance the effectiveness of song as a territorial display.

The three experiments were all of similar design. We plotted the territories of all the pairs (which were colour ringed) in a 6 hectare piece of mixed deciduous woodland² (Higgins Copse) near Oxford in early spring. In each experiment the wood was divided into three areas: control, single song and repertoire. At the start of an experiment we captured (under licence) all the resident birds in the wood during the first two or three hours after dawn on one day. The control area was left empty, the single song and repertoire areas were occupied by loudspeakers broadcasting either a single song type or a varied repertoire of songs. The broadcasting system consisted of a Uher 4000 IC tape recorder equipped with a 'Cousino' endless loop tape linked by a 15-W amplifier to four Goodman's Midax

metal horns. Each metal horn was located 50 m from the tape recorder in a different part of the 'occupied' territory. The system was powered by a 12 V battery and it operated on a time schedule controlled by a Crouzet 24 h time clock linked to a Crouzet 30 min cam timer.

The timer was set so that song was played for two minutes from each of the four loudspeakers in turn (a total of eight minutes of song). The eight minute cycle was triggered by the 24 h time clock at intervals throughout the day from dawn till dusk. During the first 3 h after dawn, the cycle was triggered once every 30 min, and for the rest of the day once an hour (simulating the natural morning peak of song). In experiments *a* and *c* (Table 2) there was one broadcasting set-up in each experimental territory; in experiment *b*, the wood was simply divided into three areas without regard to the previous territory boundaries, and the two experimental areas were occupied by a single tape recorder linked to six loudspeakers. To control for variations in attractiveness of different parts of the wood, we altered the positions of the treatment areas between experiments.

The broadcast songs were recorded from the local birds which had previously occupied territories in the wood, or from birds occupying territories in a nearby wood. We had previously made a comparison of the effectiveness in deterring invaders, of songs from within the wood and songs from nearby woods, and found that there was no difference: in four paired comparisons occupied by 'native' and 'nearby' repertoires, the following results emerged; on one occasion, the territory occupied by native songs was invaded first, on another, the other territory was invaded more quickly, and on two occasions there was no difference in latency to occupation. The repertoires used in the present experiments consisted either of local birds' song or a mixture of local and other birds' songs, and in the single song treatments we used local birds' songs.

Throughout each experiment, the pattern of reoccupation of the wood by newcomers was monitored by continuous observation from dawn till dusk. The locations of all new birds were marked on a map with reference to grid posts, and all parts of the wood were visited at least once each hour. The experiment was deemed to have ended when the

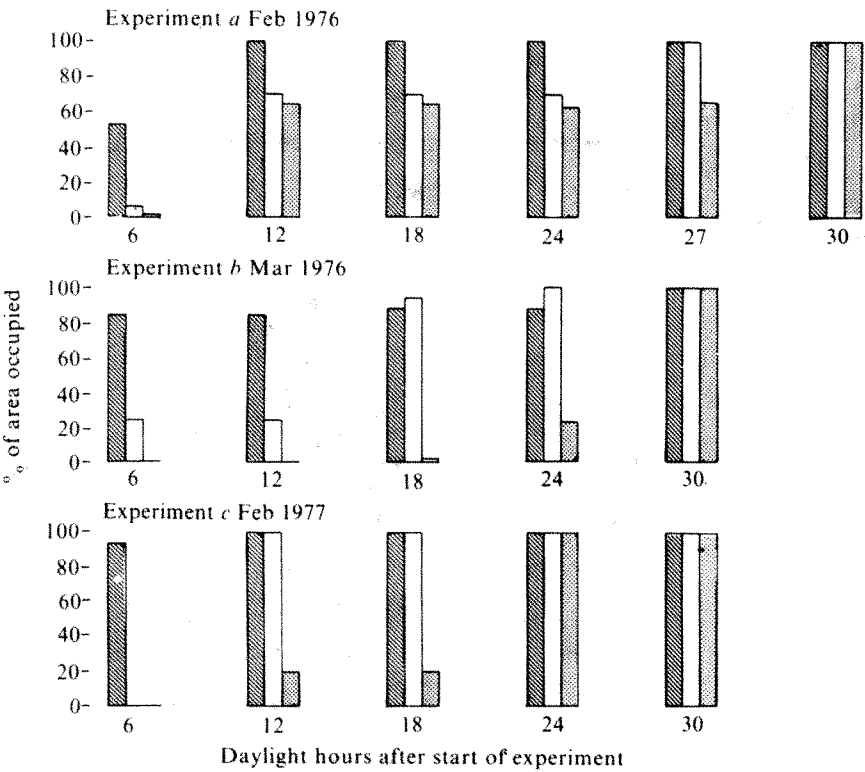


Fig. 1 A summary of the results of the three experiments. The graphs show the cumulative proportion of the three areas occupied since the start of the experiment after different numbers of daylight hours. Cross-hatched columns, control; open columns, single song; stippled columns, repertoire.

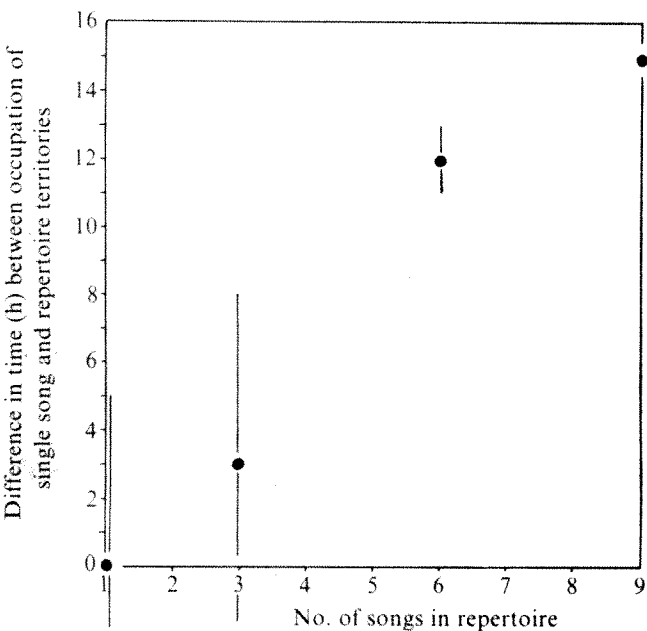


Fig. 2 The difference (in daylight hours) in the time to 90% occupation of repertoire and single song areas in relation to repertoire size. The vertical bars are standard errors. The reason for plotting the difference rather than absolute time for occupancy is that the total time span of the experiment differed between the three replicates. We took 90% of the area occupied as a criterion of 'occupation' because it was impossible to distinguish in our observations between 90% and 100% occupancy.

wood was completely occupied by new birds, at which point the speakers were turned off.

The results are summarised in Figs 1 and 2, and Fig. 3 shows an example of the reoccupation pattern over successive days. Figure 1, in which we plot the cumulative proportion of the wood occupied by new birds as a function of time, shows that in all three experiments the sequence of reoccupation of areas was: control, single song and repertoire. The three experiments were performed with repertoires of different sizes spanning the normally occurring range: 3, 9 and 6 in experiments *a*, *b* and *c*, respectively. Figure 2 shows that the difference between the time to 90% occupation of the repertoire and single song areas increases with increasing size of repertoire. We suggest that song repertoires enhance the effectiveness of song as a 'keep out' signal, and that larger repertoires are more effective than small ones. In view of this latter finding, it is surprising that the commonest repertoire sizes in our study area are 3 and 4 song types.

One or both of two mechanisms might account for our results. First, the various songs in the repertoire could be used in slightly different contexts. Three possible contexts are: time of the year, position in the territory, and the particular rival at whom the song is directed. We tested

whether or not birds use different song types at different stages in the season of territory establishment by comparing the relative frequency of use of songs during two sample periods separated by about two weeks (late January versus mid-February for three birds, and late February versus mid-March for three others). The correlation coefficient based on all six birds was highly significant ($P < 0.001$), suggesting that relative frequency of use of songs in the repertoire does not vary with season. We also did a similar correlation analysis of the relative use of different song types in different parts of an individual's territory by dividing each of the same six territories arbitrarily into north and south halves: again the correlation was highly significant ($P < 0.001$). This analysis was performed because the different songs in a repertoire might be adapted for use in the specific acoustic climate of different parts of the territory, and it is still possible that a more detailed analysis would support this idea, although our present results do not. One contextual factor which does influence the use of song types in a repertoire is the song of intruding rivals: resident birds tend to match the songs of intruders (J.K. & R.A., in preparation). Matching seems to act as a way of indicating that the song is directed at a particular intruder (who then retreats), and clearly a bird with a repertoire of songs is better able to match. This was not a major contribution to our results, however, as the loudspeakers in our experiments were not specifically programmed to match the songs of invaders.

The second mechanism which might account for our results is the so-called Beau Geste effect³. It is based on the fact that, as our experiments show, birds attempting to establish a territory use song as a cue in choosing where to settle. Reproductive success in woodland in the great tit

Fig. 3 An example of the results of one experiment (experiment *b*). The four maps show the sequence of reoccupation of the wood and the division of the wood into three treatment areas. Key: broken lines, division into treatments; large dots, new territorial pair in control area; small dots, new pair in repertoire area; cross-hatching, new pair in single song area; *a-f* represents the sequence of arrival of the new birds (*b* disappeared after day 2). Scale 6 mm, 50 m.

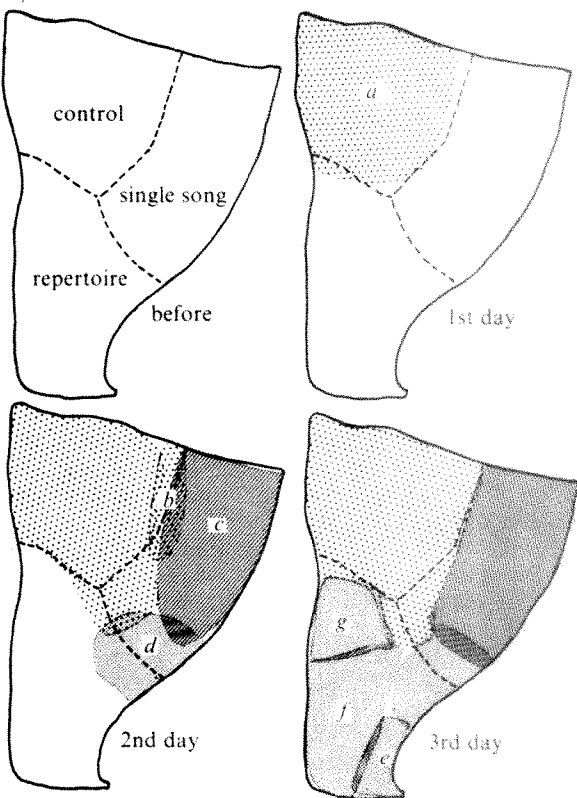


Table 2 A summary of the three experiments reported in this paper

Experiment	Date	No. of territories before			No. of pairs reoccupying the wood
		R	S	C*	
<i>a</i>	Feb 23-25 1976	3	2	3	4
<i>b</i>	Mar 15-18 1976	4†			4
<i>c</i>	Feb 15-18 1977	2	2	2	6

*Repertoire, single song, control.
†In this experiment the 3 treatments were assigned without regard to previous boundaries (see text).

Table 3 Data showing that a male is much more likely to change song perches at the same time as changing song types

Probability of changing perches N	Next song burst	
	Same song type	Different song type
	0.035	0.44
	1,755	18
	$\chi^2 = 79.95$	

The data were collected in an open parkland area (University Parks, Oxford), and changing song perches was defined as changing trees (the birds often hop about continually within a tree). The data only refer to changes in perch or song type within a period of song (that is, the bird did not pause for longer than 30 s between song bursts).

is negatively correlated with density⁶, so a settler should avoid crowded woodlands. Residents might therefore discourage invaders by singing several different songs, and creating the impression of a crowded habitat. The fact that territorial birds tend to change perches when they change song type (Table 3) is consistent with this ideal. Of course the deception we are proposing would only work to the extent that potential settlers do not correct for repertoire size.

We thank the S.R.C. for financial support, M. Dolan and D. Morris for building apparatus, and Nick Davies for helpful comments on the manuscript.

Dr M. I. Webber died in an accident at Walney on 6 June 1976.

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Tolerance of *Triticale*, wheat and rye to copper deficiency

THE aim in producing *Triticale* was to combine the hardiness and tolerance of low soil fertility of rye with the superior yield and bread-making qualities of wheat¹, but there has been little experimental evidence that triticales carry the desirable characteristics from rye². We present here evidence of the tolerance of triticales to low concentrations of available copper in soil, a condition widely associated with poor sandy soils in Australia³. Such soils may contain enough total copper for tens of thousands of crops but it is relatively unavailable to widely grown cultivars of wheat, oats and barley. In contrast, rye rarely shows a response to copper on these soils⁴⁻⁷.

Triticales are hybrids of wheat and rye and there are two major classes: hexaploids, the most promising agriculturally, are derived from crosses between diploid rye (*Secale cereale*) and tetraploid wheat (*Triticum durum*), and octoploids come from crosses between rye and hexaploid wheat (*Triticum aestivum*). In our study a hexaploid triticales and an octoploid triticales and their parent genotypes were grown in pots of copper-deficient soil with four levels of copper supplied. Chinese Spring wheat and Imperial rye are known to be the parents of the octoploid triticales⁸ but the parents of the hexaploid triticales Beagle are not known, it having resulted from crosses between many parent genotypes⁹. Accordingly, the genotypes chosen to represent the parents of the hexaploid in our study are approximate only, though

certainly, together with Chinese Spring, their response to the level of copper supplied is characteristic of their species⁴⁻⁷.

Grain yields for each of the five genotypes at each level of copper are shown in Table 1. Both wheats were extremely sensitive to the level supplied, producing no grain without supplementary copper. In contrast, rye and the hexaploid triticales were remarkably tolerant of this soil, producing as much grain in untreated soil as when copper was added. The mean grain yield of the Imperial rye was unusually low because of its poor adaptation to the environment; it matured four weeks later than the other genotypes under high temperatures and consequently grain numbers were low and grain size was small although, like grain yield, neither grain number nor size responded to copper application.

Clearly hexaploid triticales has followed its rye parentage in its tolerance of low copper supply; its yield without added copper was comparable to that of wheat in soil with the highest level of added copper. The most immediate explanation for the gross differences in response to copper supply seems to be male sterility. Copper deficiency causes male sterility in wheat¹⁰ and both pollen viability and grain number (D. T. Pearce and R.D.G., unpublished results) have shown the same pattern of response to copper supply as did grain yield (Table 1).

The difference in response of wheat and rye in copper-deficient soils is due to the ability of rye to maintain a higher concentration of copper in the shoot^{4-7,11}. Concentrations of copper in triticales seem to be intermediate between wheat and rye (unpublished results) but sufficient to maintain the plant above the threshold which prevents male sterility. Rye has a more extensive root system than wheat¹² but the higher levels of copper may be due to a more specific character than size of root system because in a study by Piper and Walkley¹¹, rye contained more copper but not more zinc.

Rye and triticales may thus be termed copper-efficient and wheat copper-inefficient. The difference in the responsiveness of octoploid and hexaploid triticales may reflect the degree of buffering of copper efficiency of the rye genome by different numbers of wheat genomes. Two genomes of tetraploid wheat retain high efficiency whereas three

Table 1 Effect of copper supply and genotype on yield of grain in a copper-deficient sandy soil

Genotype	Ploidy	Copper supply (mg per pot)			
		0	0.1	0.4	4.0
		Grain yield* (g per pot)			
<i>Triticum durum</i> cv Cocorit	Tetraploid	0	0.1	5.6	12.5
<i>Triticum aestivum</i> cv Chinese Spring	Hexaploid	0	3.5	12.9	13.7
<i>Secale cereale</i> cv Imperial	Diploid	2.1	2.4	2.4	2.1
<i>Triticale</i> cv Beagle	Hexaploid	14.3	13.8	14.3	14.5
<i>Triticale</i> Chinese Sp.-Imperial	Octoploid	4.6	6.5	7.4	7.5

*Each value is the mean of three replicates. Plants were grown in a copper-deficient siliceous sand from Tintinara, South Australia (Laffer Sand) in an evaporatively cooled glasshouse. Each pot contained 12 kg of soil which supported three plants. Appropriate amounts of nutrients N, P, K, Ca, Mg, S, Fe, B, Mn, Zn, Mo, Co and Cl were added with or without Cu. Of the genotypes, only wheat plants failed to grow normally in the soil without added Cu, showing characteristic symptoms of wilting, delayed maturity and failure to set grain¹⁰. The copper responses of both wheats and the octoploid triticales are significant at $P = 0.001$.

genomes of hexaploid wheat reduce the efficiency to a position intermediate to hexaploid triticale and hexaploid wheat.

Several nutritional characters have been shown to be under single-gene control, for example, iron efficiency in soybeans¹³ and boron efficiency in tomato¹⁴, and this may also be the case for copper. Pugsley (personal communication) in a hybridisation study of copper-efficient oats (*Avena strigosa*) and copper-inefficient oats (*A. sativa*) has found copper efficiency to be controlled by one or two genes. Triticale cultivar Beagle used in my study contains the complete rye genome of seven chromosome pairs⁹, but studies in progress suggest that copper efficiency is carried on a single rye chromosome, and if this is so, it should be possible to translocate the relevant part of the chromosome to wheat to produce a copper-efficient line of wheat.

Copper efficiency from rye is transferable to triticale and the potential of this new crop for marginal lands deserves further examination. The mechanism of enhanced copper utilisation is important too, for if it is linked with the morphology of the root system, it may influence the utilisation of other immobile soil nutrients.

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Received 18 November; accepted 18 December 1977.

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Activation of an endogenous retrovirus from *Tupaia* (tree shrew)

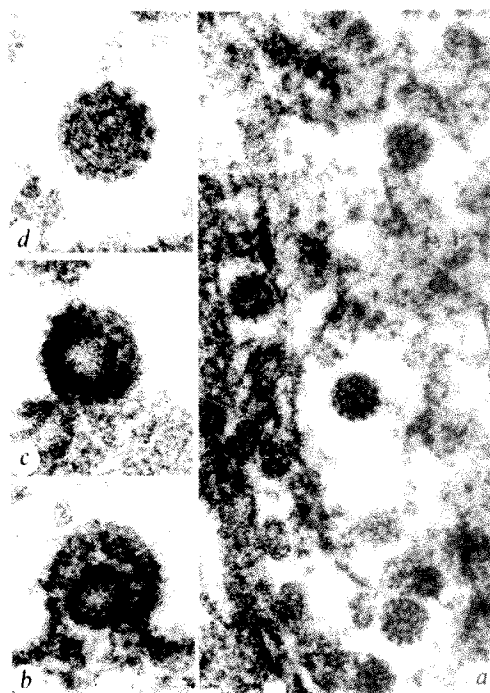
TYPE-C RNA viruses have been isolated from tissues and cell lines of various mammalian species, including mice^{1,2}, domestic cats³, pigs^{4,5}, woolly monkeys^{6,7}, gibbons⁸ and squirrel monkeys⁹. The viruses from woolly monkeys seem to be horizontally transmitted as infectious agents which cause naturally occurring tumours in gibbons⁷. Apart from the known aetiological role of certain oncornaviruses in the formation of neoplasms, there has been increasing interest in the isolation of mammalian retroviruses and their evolutionary relatedness to different species. Experimental evidence suggests that during evolution an endogenous virus crossed from one species to another¹⁰. *Tupaia* (the tree shrew), a member of the family Tupaiidae, is regarded as one of the most primitive living prosimians, bridging the gap between insectivores and primates^{11,12}. We report here the detection and activation, *in vitro* and *in vivo*, of an endogenous retrovirus from *Tupaia*. This opens up the possibility of a direct comparison of the *Tupaia* retrovirus with type-C viruses from rodents and primates.

Reports^{13–15} on type-C RNA viruses in placentae of normal, healthy Sprague-Dawley rats initiated a search for an analogous *Tupaia* virus in placentae of healthy, untreated animals and others treated with iododeoxyuridine (IUDR) 24 h before the end of pregnancy. We used *Tupaia belangeri* Baf: BIAS (Baf designates the breeder, the Battelle-Institut, Frankfurt and BIAS is the name of the stock used¹⁶). Placentae were

removed aseptically from untreated, healthy, pregnant *Tupaia* at full term and prepared for electron microscopy. Figure 1 shows an electron photomicrograph of virions, which in size and structure are morphologically similar to type-C virus particles. Figure 1b shows a budding virus particle; some virions have spike-like structures consistent with reports that the ultrastructure of type-C virus particles varies considerably^{17,18}.

We took a more direct approach by establishing *Tupaia* fibroblasts from skin of single embryos or juvenile animals in tissue culture. Some cultures were treated with IUDR to activate retrovirus. We describe here the experiments with the cell cultures derived from the second embryo of one animal (no. 458, TEF-458-2). The cells were established and propagated in tissue culture as before¹⁹. They were grown at 37 °C and treated with IUDR in the replication log phase of cell growth. After treatment with IUDR (50–200 µg ml⁻¹) for 24 h several clones were isolated and established in tissue culture. Treatment of *Tupaia* fibroblasts with 200 µg IUDR per ml of medium had no highly toxic effect within 24 h. The plating efficiency of the treated cells (measured according to Ham²⁰) decreased to 17% that of untreated cells and was 22.5%. The supernatants of the different cell clones were assayed for reverse transcriptase activity^{21,22}. Poly (rA)·(dT)₁₄ served as template/primer; poly (dA)·(dT)₁₄ was used as a control²³. The incorporation of ³H-dTMP was maximal in the presence of 0.5 mM Mn²⁺ as divalent cation (Fig. 2). In the presence of poly (dA)·oligo (dT) only small or negligible levels of RNA-directed DNA polymerase (RDDP) activity were observed. RDDP activity detectable with poly (rA)·oligo (dT) was inhibited by 2 mM inorganic phosphate, which is characteristic for mammalian virus DNA polymerase²⁴. Low levels of ³H-dTMP incorporation were observed in supernatants of *Tupaia* cell cultures not treated with IUDR, whereas an increase

Fig. 1 Electron micrographs of ultrathin sections of *Tupaia belangeri* placenta. The placenta tissue was fixed in 2% glutaraldehyde, buffered with 0.05 M sodium cacodylate, pH 7.2, in the cold for 20 min, followed by several washes with cold buffer and osmication in the cold (2% osmium tetroxide). The material was dehydrated through graded ethanol solutions and embedded in Epon 812. *a*, Virus particles in *Tupaia* placenta (the bar represents 200 nm). *b*, Virus particle budding like type-C virus from *Tupaia* placenta (the bar represents 100 nm); the centrally located nucleocapsid is in direct contact with viral envelope (the bar represents 100 nm). *c*, The cylindrical particle has an internal structure similar to that of the immature type-C particle. *d*, Extracellular, immature virus particle (the bar represents 100 nm).



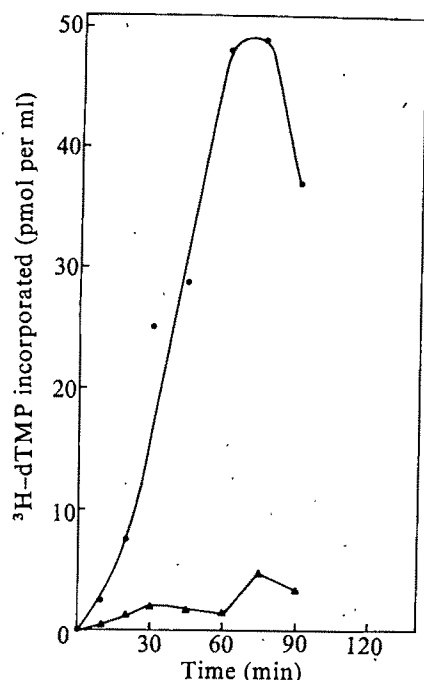


Fig. 2 Kinetics of polymer synthesis with reverse transcriptase from supernatants of TEF-458-2-J-2 cell cultures using poly (rA)·oligo (dT) (●) or poly (dA)·oligo (dT) (▲) as template/primer. The reaction mixtures (0.10 ml) consisted of 50 mM Tris-HCl, pH 8.1, 30 mM KCl, 0.5 mM MnCl₂, 1 mM dithiothreitol, 0.08 mM dTTP including ³H-dTTP (Radiochemicals Centre, Amersham, specific activity 62 Ci mmol⁻¹), poly (rA)·(dT)₁₄ at 48 μM (Collaborative Research, Waltham, Massachusetts). Reactions were started by the addition of 60 μl viral suspension (disrupted by Nonidet P-40 for 10 min at 24 °C) to the polymerisation mixtures. Reactions were carried out at 37 °C and monitored as described before²³.

in RDDP activity was observed routinely in cultures treated with halogenated pyrimidines (Table 1). All cell cultures used for the DNA polymerase assays (Table 1) were of either the same or nearly the same passage level.

To activate retrovirus *in vivo* from *Tupaia*, a pregnant animal (no. 944, 205 g body weight) was injected intraperitoneally with IUdR (10 μg per g body weight) on the 35th day of gestation—the normal period of gestation being 44 ± 1 d. Embryo no. 3 was removed aseptically by caesarean section 24 h later, and skin fibroblasts were explanted and

established in culture. RDDP activity (Table 1) revealed an increase in ³H-dTMP incorporation for TEF-944-3-J compared with the low levels found in supernatants of TEF-944-1 cultures derived from embryo no. 1 which had been removed before the injection of the mother animal no. 944 with IUdR.

To detect viral RNA we used ³H-uridine (specific activity 58 Ci mmol⁻¹). After phenol extraction²⁵ a peak of radioactivity banded at 70S after 5–20% sucrose gradient centrifugation (RNA from Gross strain of MuLV served as marker). When subjected to heat denaturation (3 min at 100 °C with 2% sodium dodecyl sulphate) the 70S RNA was converted into a broad peak centred around 35S. Furthermore, ³H-uridine-labelled virions from supernatants of three *Tupaia* cell cultures (TEF-944-3-J, TEF-458-2-J-2 and TEF-458-2-J-2-J) were isolated after purification by low and high speed centrifugation and banded in 15–55% neutral sucrose gradient. The activity peak banded at a density of 1.15–1.17 g cm⁻³—characteristic for retroviruses²⁶ (Fig. 3).

In a host range study, the isolated *Tupaia* retrovirus (TRV-1) was inoculated into human embryonic lung fibroblasts (HELFI)²⁷, human foreskin fibroblasts (HFF-3)²⁸, marmoset skin fibroblasts (HF)²⁹, mink lung cells (Mv1Lu)³⁰ and dog thymus cells (Fcf2th)³⁰. RDDP activity was detected in culture fluid from inoculated canine cells only at a low level. Cocultivation of isolated *Tupaia* cell clones (TEF-944-3-J, TEF-458-2-J-2 and TEF-458-2-J-2-J) with XC cells³¹ did not result in syncytia formation. Cocultivation of XC cells with Fcf2th cells infected with TRV-1 resulted in the formation of multinucleated giant cells containing 10–20 nuclei. The lack of formation of giant cells after cultivation of producer cell clones (TEF-458-2-J-2, TEF-944-3-J and TEF-458-2-J-2-J) with XC cells could indicate that TRV-1 is not of murine origin. However, the lack of induction of XC syncytia does not rule out a murine origin for TRV-1, because some murine leukaemia viruses do not induce XC syncytia³². Cytotoxicity tests with *Tupaia* producer cell clones and anti SSV-1 sera gave negative results. This suggests that SSV-1-specific surface receptors are not present in the tree shrew cell clones.

The absence of RDDP activity in normal *Tupaia* cells, and the inducibility by IUdR of a retrovirus from three different tree shrew cell cultures, suggest that TRV-1 is an endogenous retrovirus. Our data represent the first isolation (as far as we know) of an oncornavirus in the primitive prosimian *Tupaia*.

This work was supported in part by the Deutsche Forschungsgemeinschaft, Sonderforschungsbereich 136. We thank Dr

Table 1 Reverse transcriptase activity of supernatants of normal and IUdR-treated *Tupaia* skin fibroblast cell cultures

Code no. of animal/no. of embryo	Label of culture	No. of tissue culture passages	Treatment with IUdR	³ H-dTMP incorporated (pmol per μg protein)*	³ H-dTMP incorporated (pmol per μg protein)†	(rA) (dA)
458/2	TEF-458-2	2	—	0.12	0.19	0.63
		3–7	—	0.10–0.46	0.15–0.65	0.67–0.71
		17	—	0.18	0.24	0.75
		25	—	0.43	0.61	0.70
	TEF-458-2-J-2	8	+	4.22	0.19	22.2
		11	+	6.19	0.16	38.7
		13	+	5.14	0.22	23.4
	TEF-458-2-J-2-J	8	+	8.57	0.21	40.8
		11	+	7.86	0.17	46.2
		13	+	5.74	0.25	22.9
944/1	TEF-944-1	2	—	0.16	0.20	0.8
		3–5	—	0.19–0.66	0.20–0.62	0.95–1.0
944/3	TEF-944-3-J	2	+	2.11	0.26	8.2
		5	+	5.75	0.20	28.8

*With poly (rA)·oligo(dT) as template/primer.

†With poly (dA)·oligo(dT) as template/primer.

Figures show incorporation of ³H-dTMP (pmol per 30 min) at 37 °C into acid-insoluble product. Assays were performed as described in the legend to Fig. 2. Protein was determined according to Lowry's³³ method.

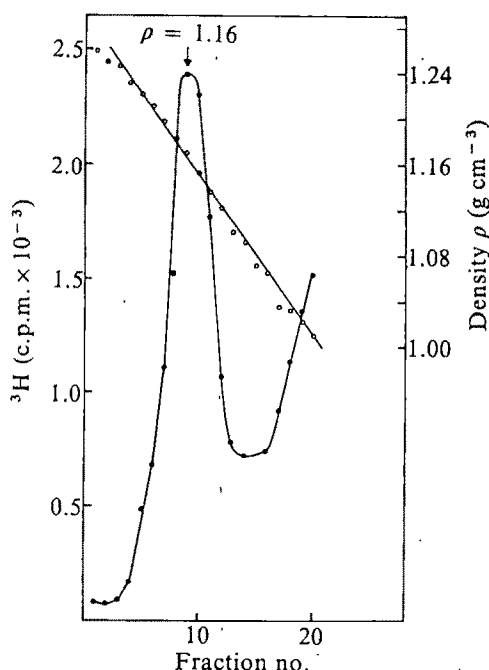


Fig. 3 Equilibrium gradient centrifugation of TRV-1 virions. Culture fluids from TEF-458-2-J-2 cells incubated with ^3H -uridine ($28 \mu\text{Ci ml}^{-1}$) were clarified by centrifuging at $1,000g$ for 20 min and centrifuged through a 5% (w/v) cushion of sucrose for 60 min at $25,000 \text{ r.p.m.}$ in a SW27 rotor. The pellet was resuspended in 0.1 M NaCl , 10 mM Tris-HCl , $\text{pH } 7.2$, and 1 mM EDTA and layered on a 5-ml linear 30–60% sucrose gradient in the same buffer. It was then centrifuged at $50,000 \text{ r.p.m.}$ in a SW65 rotor for 4 h at 4°C . The gradient was fractionated and each fraction was assayed for acid-precipitable radioactivity (●) and density (○).

F. Deinhardt for valuable discussion and critical comments and for XC, HFF-3 and HF cells; Dr C. Bergholz for Mv1Lu and Fc2th cells; Dr G. Hunsmann for the cytotoxicity test; Dr W. W. Franke for comments on the electron micrographs; Dr A. Schwaier for advice on the animal experiments, and Ms Ulrika Gramlich and Ms Renate Schulz for technical assistance.

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Received 19 September; accepted 25 November 1977.

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5'-AMP is a direct precursor of cytokinin in *Dictyostelium discoideum*

ISOPENTENYLADENINE ($i^6\text{Ade}$) and closely related derivatives occur naturally either as free bases or as constituents of tRNA^{1,2}. Although these cytokinins have been detected in various organisms, it is still not clear whether they are produced by the degradation of tRNA or whether they are synthesised by some pathway not involving tRNA. A spore germination inhibitor, discadenine, isolated from *Dictyostelium discoideum* was found to be an $i^6\text{Ade}$ derivative with a 3-amino-3-carboxypropyl group in the 3 position³—the cells contained a large quantity of free $i^6\text{Ade}$, presumably a precursor of discadenine⁴.

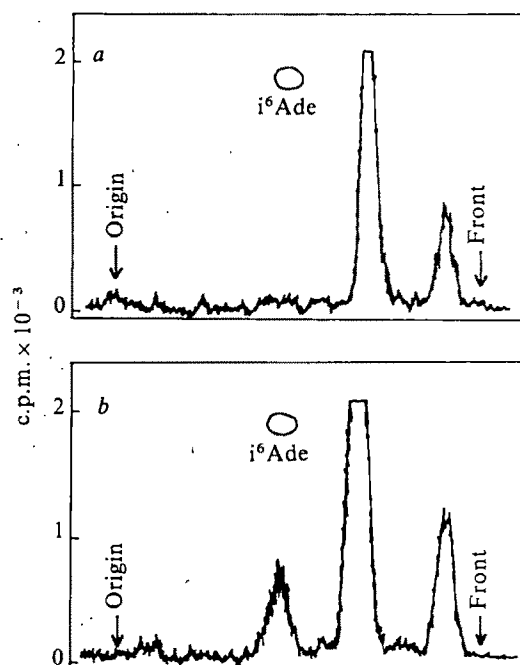


Fig. 1 Thin-layer chromatography of the products synthesised *in vitro* using ^{14}C -isopentenylpyrophosphate in the absence (a) or presence (b) of the supernatant of nondialysed, boiled S-100. The reaction mixture contained 20 mM Tris-HCl ($\text{pH } 7.5$), 5 mM magnesium acetate, $0.05 \mu\text{Ci}$ of ^{14}C - Δ^3 -isopentenylpyrophosphate (57 mCi mmol^{-1} ; Radiochemical Centre), 0.5 mg of dialysed S-100 extract and $10 \mu\text{l}$ of the supernatant of nondialysed, boiled S-100 in a final volume of $100 \mu\text{l}$. After 60 min incubation at 27°C , $150 \mu\text{l}$ of ethylacetate was added and the mixture was then vigorously shaken and centrifuged. The resultant upper layer was spotted on a plate of silica gel (Merck) and developed (10 cm) with chloroform-acetic acid (9:1, v/v). Chromatograms were scanned with a radiochromatogram scanner (Packard 385). Slit width was 2.5 mm . The S-100 extract was prepared from late culmination cells of *D. discoideum* strain NC-4 as follows; 1 g of cells were ground with 2 g of alumina, and extracted with 5 ml of buffer A (10 mM Tris-HCl , $\text{pH } 8.0$, 10 mM magnesium acetate, 60 mM KCl , 1 mM EDTA , 6 mM 2-mercaptoethanol and 10% glycerol). The extract was centrifuged at $13,000g$ for 30 min and the resulting supernatant was then further centrifuged at $156,000g$ for 60 min. The supernatant obtained was dialysed overnight against two changes of buffer A (300 ml each). The supernatant of nondialysed, boiled S-100 was prepared by boiling the nondialysed S-100 ($25 \text{ mg protein ml}^{-1}$) for 2 min and centrifugation.

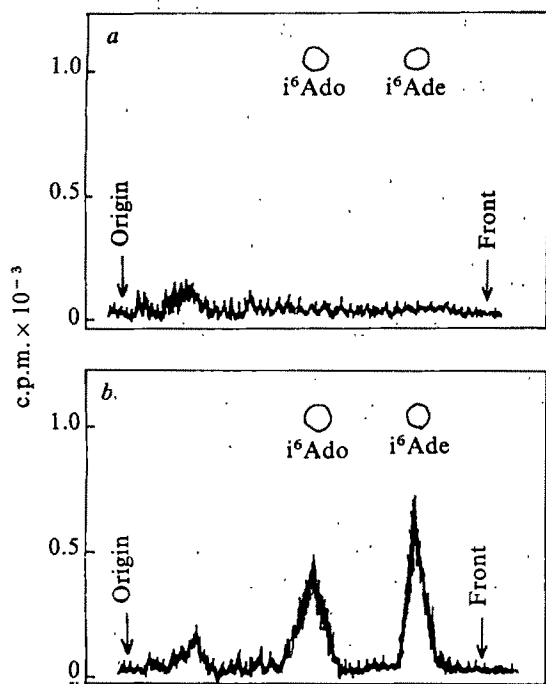
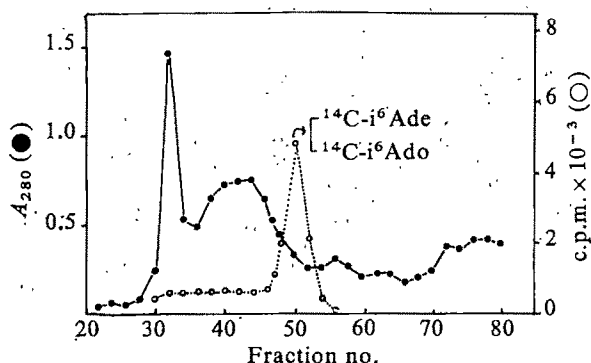


Fig. 2 Thin-layer chromatography of the products synthesised *in vitro* using ^{14}C -5'-AMP in the absence (a) or presence (b) of Δ^2 -isopentenylpyrophosphate. The reaction mixture contained 20 mM Tris-HCl (pH 7.5), 5 mM magnesium acetate, 0.05 μCi of $[\text{U-}^{14}\text{C}]$ 5'-AMP (538 mCi mmol $^{-1}$, Radiochemical Centre), 0.5 mM Δ^2 -isopentenylpyrophosphate and 0.5 mg of dialysed S-100 in a final volume of 100 μl . After 60 min incubation at 27 $^{\circ}\text{C}$, the reaction mixture was extracted with ethylacetate. The extract was subjected to thin-layer chromatography in chloroform-acetic acid (1:1, v/v) and then scanned as described in the legend to Fig. 1. Δ^2 -Isopentenylpyrophosphate was synthesised chemically by the method of Kandutsch *et al.*⁵.

These results led us to investigate the cell-free biosynthesis of $i^6\text{Ade}$ in this organism. We report here the existence of a cytokinin biosynthetic pathway not related to tRNA degradation, showing that 5'-AMP is the actual acceptor molecule for the isopentenyl group. Our findings suggest that the biosynthesis of cytokinin in higher plants may proceed in a similar fashion.

We first synthesised $i^6\text{Ade}$ in reaction mixture containing ^{14}C - Δ^2 -isopentenylpyrophosphate and crude extracts (S-100) of

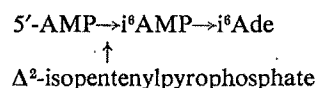
Fig. 3 Gel filtration of the S-100 extract on a Sephacryl S-200 column. S-100 extract, containing 15 mg of protein, was applied to a Sephacryl S-200 column (1.5 \times 70 cm) which had previously been equilibrated with buffer B (50 mM Tris-HCl pH 7.5, 1 mM EDTA, 6 mM 2-mercaptoethanol and 10% glycerol). Elution was carried out with buffer B and 1.5 ml fractions were collected. $i^6\text{Ade}$ synthesis was assayed with the reaction mixture described in Fig. 2, except that 20 μl of each fraction was used instead of S-100. After incubation at 27 $^{\circ}\text{C}$ for 60 min, the reaction mixture was extracted with 150 μl of ethylacetate and 30 μl of the ethylacetate layer was counted in a Beckman, Model LS-200B, liquid scintillation counter in toluene-based scintillator.



D. discoideum at the stage of late culmination. As shown in Fig. 1, dialysed S-100 catalysed the formation of a radioactive material that coincided in position with the authentic $i^6\text{Ade}$ spot only when the supernatant of nondialysed, boiled S-100 was added. Essentially similar results were obtained with another solvent system (chloroform : methanol, 8 : 2, v/v). These findings suggest that $i^6\text{Ade}$ was in fact synthesised and that a heat-stable, dialysable substance was the actual acceptor of the isopentenyl group. Tests on various compounds related to adenine showed that adenine nucleotides such as 5'-AMP, ADP, ATP and 3', 5'-cyclic AMP, but not adenine or adenosine, could replace the supernatant of nondialysed, boiled S-100 in synthesis of $i^6\text{Ade}$ (data not shown). These results were confirmed in another kind of reaction using ^{14}C -labelled compounds as acceptors and unlabelled Δ^2 -isopentenylpyrophosphate as donor; addition of 5'-AMP, ADP, ATP or cyclic AMP led to formation of $i^6\text{Ade}$, while addition of Ade or Ado did not. An example is shown in Fig. 2. In this experiment, isopentenyladenosine ($i^6\text{Ado}$) was also produced. Of the adenine nucleotides tested, 5'-AMP seemed to be the direct acceptor of isopentenyl group, because addition of unlabelled 5'-AMP strongly inhibited the formation of $i^6\text{Ade}$ when ^{14}C -ADP, ^{14}C -ATP or ^{14}C -cyclic AMP was used, whereas addition of cold ADP, ATP or cAMP only slightly inhibited the synthesis of ^{14}C - $i^6\text{Ade}$ from ^{14}C -AMP (data not shown).

To confirm the identity of the direct substrate in synthesis of $i^6\text{Ade}$, S-100 was purified by gel filtration on a Sephacryl S-200 column (Fig. 3), and each fraction was assayed for formation of a radioactive product extracted with ethylacetate, using ^{14}C -AMP and unlabelled Δ^2 -isopentenylpyrophosphate as substrates. The activity appeared in a central fraction as a sharp peak. The radioactive products were found to be a mixture of ^{14}C - $i^6\text{Ade}$ and ^{14}C - $i^6\text{Ado}$. With this fraction as enzyme source, ^{14}C -cyclic AMP or ^{14}C -ATP was not utilised for the synthesis of $i^6\text{Ade}$ and $i^6\text{Ado}$, indicating that 5'-AMP is the direct acceptor of the isopentenyl group. It is very likely that the first reaction product is isopentenyl AMP ($i^6\text{AMP}$) and that this is converted to $i^6\text{Ado}$ and $i^6\text{Ade}$. To confirm this possibility, the peak fraction in Fig. 3 was further purified by phosphocellulose column chromatography. Fractions were assayed by analysing the total reaction products by thin-layer chromatography without previous treatment of the mixture with ethylacetate. As shown in Fig. 4, ^{32}P - $i^6\text{AMP}$ was in fact synthesised from ^{32}P -AMP. Radioactive $i^6\text{Ade}$ was also synthesised by fractions 18 to 20 using ^{14}C -AMP instead of ^{32}P -AMP without formation of ^{14}C - $i^6\text{Ade}$ or ^{14}C - $i^6\text{Ado}$ (data not shown). These results show that the first reaction product is $i^6\text{AMP}$. Because scarcely any $i^6\text{Ade}$ or $i^6\text{Ado}$ was detected, the enzymes which convert $i^6\text{AMP}$ to these products were thought to be separated from the enzyme synthesising $i^6\text{AMP}$.

It can be concluded from these studies that the overall pathway for $i^6\text{Ade}$ synthesis is as follows:



Further investigations are needed to determine whether $i^6\text{Ade}$ is formed directly from $i^6\text{AMP}$ or through $i^6\text{Ado}$. $i^6\text{AMP}$ was scarcely detected in the reaction mixture using a crude extract, due to its quick degradation to $i^6\text{Ade}$. This indicates that the cells contain exclusively $i^6\text{Ade}$, but not its nucleotide. In this respect it should be noted that cytokinin activity of $i^6\text{Ade}$ is almost 2 orders of magnitude higher than that of $i^6\text{Ado}$, $i^6\text{AMP}$ and cyclic $i^6\text{AMP}$, when the activity was determined by the tobacco assay⁸.

It is well known that cyclic AMP acts as a chemotactic factor causing aggregation of *D. discoideum* cells^{9,10}. 5'-AMP, which is the first substrate for $i^6\text{Ade}$ biosynthesis, is formed by hydrolysis of cyclic AMP by cyclic AMP phosphodiesterase, and that cyclic AMP, $i^6\text{Ade}$ and discadenine are all compounds related to adenine. Thus these compounds may be closely inter-

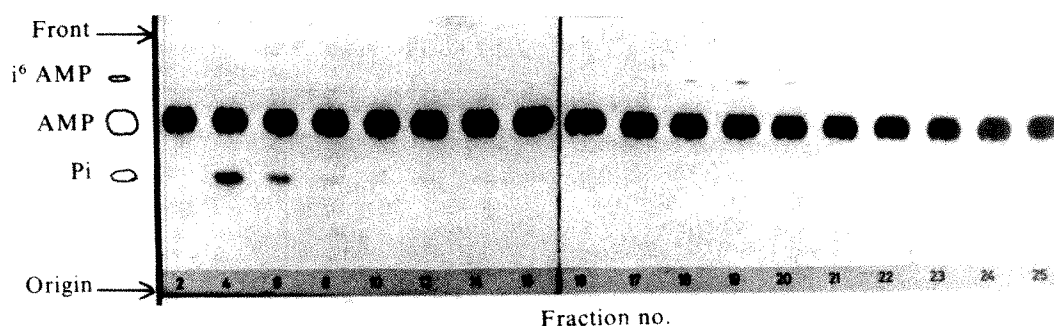


Fig. 4 Autoradiography of a thin-layer chromatogram of the products synthesised by the fractions obtained by phosphocellulose column chromatography. The peak fractions from Sephacryl S-200 (fractions 49–51) were pooled and applied to a phosphocellulose column (Whatman P11, 0.7×12 cm) which had previously been equilibrated with buffer B. Elution was carried out with a linear gradient of KCl (0–0.5 M; total volume of the gradient, 30 ml) and 1-ml fractions were collected. The reaction mixture contained 20 mM Tris-HCl pH 7.5, 5 mM magnesium acetate, 0.5 mM Δ^2 -isopentenylpyrophosphate 0.2 μ Ci of 32 P-5'-AMP (500 mCi mmol $^{-1}$) and 20 μ l of phosphocellulose column fraction in a final volume of 50 μ l. After incubation at 27 °C for 60 min, reaction mixture was cooled in ice and 5- μ l portions were spotted directly onto thin-layer plates coated with cellulose powder (20 \times 20 cm, Funakoshi). Material was developed using isobutyric acid–0.5 M NH_4OH (5:3, v/v). 32 P-5'-AMP was synthesised by the method of Symons⁶. Unlabelled i^6 AMP used as marker was synthesised by the method of Yoshikawa *et al.*⁷.

related, with important roles in regulation of the development of *D. discoideum*. In *D. discoideum*, i^6 Ade is an intermediate in discadenine biosynthesis, but it probably also has some important physiological activity itself, like cytokinin in higher plants.

We thank Dr T. Sasaki for helpful discussions.

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Received 25 July; accepted 22 November 1977.

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X chromosome inactivation in diploid parthenogenetic mouse embryos

THE mechanism by which one X chromosome in normal cells of female mammals becomes inactive while the other, apparently identical, chromosome remains active is not known. In kangaroos the paternally derived X chromosome is preferentially inactivated in most tissues¹, and in mice and rats similar preferential inactivation of the paternal X chromosome occurs in the cells of the extraembryonic membranes^{2,3}. Paternal X inactivation in kangaroos led Cooper⁴ to postulate that passage of the X chromosome through male gametogenesis was an important factor in its inactivation. He⁴ and Brown and Chandra⁵ suggested that paternal X inactivation was a primitive form and that random X inactivation as seen in adult eutherian mammals had evolved from it. Brown and Chandra further suggested that passage of one chromosome set through male gametogenesis or fertilisation led to chromosomal imprinting. A locus concerned in the control of X chromosome activity was postulated to become inactive when imprinted, and one active copy of this gene could maintain the activity of one

X chromosome. In marsupials this gene was postulated to lie on the X chromosome itself, but on an autosome in eutherians. Thus, in eutherians the number of active X chromosomes in any animal should be equal to the number of maternally derived (and therefore non-imprinted) autosome sets. In this context it is interesting to study X inactivation in artificially formed parthenogenetic embryos, in which all chromosomes are of maternal origin. We report here that diploid parthenogenetic mouse embryos, at the late egg cylinder stage, showed a single late replicating chromosome, indicating that X inactivation had occurred normally. Because in the production of these embryos polar body formation had been suppressed, it seems that neither passage of chromosomes through male gametogenesis, nor fertilisation, nor probably the effects of peripheral egg cytoplasm are required for normal X inactivation in the mouse.

In the normal mouse embryo X chromosome inactivation is thought to occur at the late blastocyst stage⁶, and a late replicating X chromosome can be recognised at about the 5-d⁷ to 7-d stage⁸. Thus, studies of DNA replication in parthenogenetic embryos at the late egg cylinder stage should indicate whether normal X inactivation has occurred.

Experimental parthenogenetic mouse embryos have been produced by various means⁹, and Kaufman *et al.*¹⁰ have achieved development of a high proportion of diploid parthenogenotes to the egg cylinder stage or later. We have now studied DNA replication by ³H-thymidine labelling at the late egg cylinder stage in diploid parthenogenetic embryos produced by their method. Eggs were activated by culturing cumulus masses containing recently ovulated oocytes for 5–6 h in medium lacking calcium and magnesium salts. Then the cumulus cells were removed with hyaluronidase, and the successfully activated presumptive diploid eggs (which developed two pronuclei after suppression of second polar body formation or extrusion) were cultured for a further 90 h in normal embryo culture medium, by which time approximately 60% had reached the blastocyst stage. Blastocysts were then transferred to pseudopregnant recipient females, which had been mated to proven sterile vasectomised males 2.5 d earlier. Recipients were ovariectomised bilaterally at the time of transfer of blastocysts, and pregnancy was maintained by exogenous hormones, so that the time of implantation could be controlled. Implantation began about 24 h after the first of a series of oestradiol injections, which were started 6 d after blastocyst transfer. Females were killed after 4 d of injections when the embryos were expected to be at a stage equivalent to about day 7.5–8.5 of normal development. The embryos were dissected free from the decidua and embryonic membranes, and placed in Eagle's medium, supplemented with foetal calf serum (20%) at 37 °C.

In the mouse, because of its many segments of constitutive heterochromatin, the asynchronously replicating X chromosome is more readily detectable by its failure to take up ³H-thymidine early in the S period, than by its excess labelling at the end of S^{8,11}. We therefore attempted to study chromosome replication patterns early in S. Embryos were allowed to incorporate ³H-thymidine (final concentration 5 μ Ci ml⁻¹; specific activity 6 Ci mmol⁻¹) for 30 min at 37 °C, followed immediately by three washings with unsupplemented medium, and a chase of medium containing a 100-fold excess of unlabelled thymidine for 10 h. Colchicine, at a final concentration of 1 μ g ml⁻¹, was present during the final 2 h of the 10-h incubation. Air-dried slides were prepared according to a modification of the method of Wroblewska and Dyban¹² and autoradiographs were prepared.

Of six embryos studied four yielded scorable mitoses. Cells with 40 chromosomes were scored for uptake of label, and for presence or absence of a single large unlabelled chromosome, presumed to be an X chromosome. In all four embryos some cells showed a clear asynchronously replicating chromosome (Table 1). In all cells this was a large chromosome, of approximately the expected size of an X chromosome (Fig. 1). In most cases the asynchronous chromosome was unlabelled in an otherwise fully labelled cell (Fig. 1a, b), but in a few cells there was a single heavily labelled chromosome in a lightly labelled set (Fig. 1c). In addition some interphase cells showed an apparent X chromatin body (Fig. 1d).

The proportion of cells showing any label varied somewhat (from 17 to 42%) among the embryos, but the proportion of labelled cells with an asynchronous chromosome

Table 1 Cells with asynchronous presumed X chromosomes in diploid parthenogenetic mouse embryos

Embryo no.	Cells examined	Cells labelled (%)	Labelled cells with asynchronous chromosome (%)
1	107	39 (36)	25* (64)
2	87	27 (31)	15* (56)
3	46	8 (17)	5 (63)
4	12	5 (42)	4 (80)
Total	252	79 (31)	49 (62)

* A few cells (four in embryo 1 and two in embryo 2) showed a late replication pattern, that is a single heavily labelled chromosome in an otherwise lightly labelled cell.

showed reasonable consistency, with a mean of 62% (range 56–80%). The failure to detect an asynchronous chromosome in the remaining cells does not of course mean that one was lacking, because the cell cycle times may have been variable, with some cells being at mid-S, and therefore unsuitable for showing asynchronous replication, at the time of the ³H-thymidine pulse. The finding of a few cells with a replication pattern characteristic of late S emphasises this point.

Our interpretation is thus that the asynchronously replicating chromosome seen in all four embryos was a late replicating (and therefore inactive) X chromosome. Clearly differentiation of two X chromosomes within a cell can take place in embryonic development without the need for fertilisation or for passage of either chromosome, or indeed any autosome, through male gametogenesis.

A similar conclusion was reached by McCaw and Latt¹³ who found late replicating X chromosomes in human

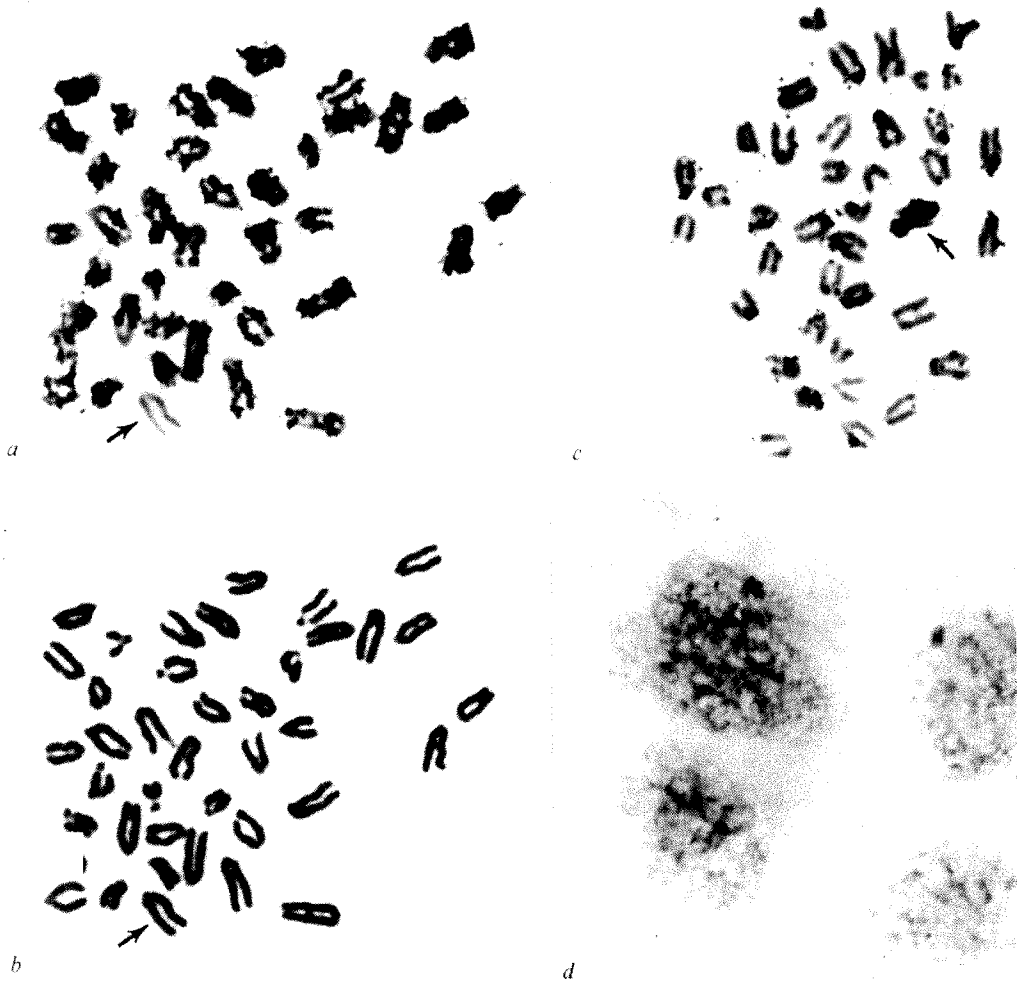


Fig. 1 a–c, Autoradiographs of cells from diploid parthenogenetic mouse embryos. a, Single unlabelled chromosome in heavily labelled cell. b, Same cell after removal of silver grains. c, Single heavily labelled chromosome in otherwise lightly labelled cell (characteristic late-S replication pattern). d, Apparent X chromatin body in some interphase nuclei. Ilford Nuclear Research emulsion, gel form L4, was used for autoradiography. Slides were exposed for 3 weeks at room temperature, developed in Amidol developer, fixed and stained with Giemsa. Silver grains were removed by transferring slides through xylene and graded alcohols to potassium ferricyanide, sodium thiosulphate and distilled water.

ovarian teratomata, which arise by parthenogenetic growth of germ cells¹⁴, and by Linder and Power¹⁴ on the basis of X-linked gene expression in such teratomata. Our work strengthens the finding by dealing with embryos which were undergoing fairly normal development (up to the relevant stage), rather than teratomata in which the genetic programming might be in some way disturbed. Thus, these results are inconsistent with the expectation from Brown and Chandra's hypothesis that the number of active X chromosomes in any cell should be equal to the number of maternally derived autosome sets. In their development¹⁵ of the original hypothesis they reconciled the X inactivation seen in certain human ovarian teratomata and a diploid/triploid mosaic by postulating that chromosomal imprinting occurred not during male gametogenesis but during passage of chromosomes through the egg cytoplasm. Normally this would occur at fertilisation, but in the cases mentioned the effect was produced by re-entry of the polar body. Our embryos were inconsistent even with this, because the parthenogenetic activation was produced in combination with suppression of polar body formation. Further evidence against Chandra and Brown's ideas has come from human triploid foetuses one of which had two active X chromosomes although only a single autosome set from the mother¹⁶ and another which had a single active X chromosome but two haploid sets from the mother¹⁷.

Thus we conclude that in eutherian mammals there is no evidence that chromosomal imprinting is required for the initiation of X-chromosome inactivation. This does not, of course, exclude the possibility of imprinting in normal embryogenesis. Preferential inactivation of the paternally derived X chromosome (manifested by late replication as detected by acridine orange fluorescence after BUdR incorporation) has been found in the extraembryonic membranes but not the foetus itself of both the mouse and the rat^{2,3}. Thus it seems probable that in eutherians, as in marsupials, imprinting of the paternally derived X chromosome does occur, but that the cells of the embryo proper escape from imprinting before X inactivation¹⁸. One might expect therefore that in parthenogenetic embryos X inactivation would be in some way abnormal in the extraembryonic membranes. Further work is necessary to test this point.

M.H.K. thanks Mrs S. C. Barton and Dr M. A. H. Surani for help. The work was supported by a grant from the Ford Foundation.

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Received 6 October; accepted 16 December 1977.

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Localisation and stimulation of prostacyclin production in vascular cells

PROSTACYCLIN (PGI₂) was reported by Vane and co-workers as a novel short-lived metabolite of prostaglandin endoperoxides (PGG₂ and PGH₂) which inhibited platelet aggregation¹. Subsequent studies have demonstrated that PGI₂ is the most potent inhibitor of platelet aggregation so far described, acting by stimulating platelet adenylate cyclase^{2–4}. PGI₂ is produced by isolated blood vessel segments⁵ and is therefore likely to be of great importance in the maintenance of vascular homeostasis. The cellular localisation and regulation of PGI₂ synthesis have not previously been established, although it has been proposed that the main source of PGI₂ is through pro-aggregatory prostaglandin endoperoxides released from platelets being enzymatically converted to PGI₂ by vascular endothelial cells^{5,6}. We show here that pig aortic endothelial cells, but not aortic smooth muscle cells or fibroblasts, synthesise PGI₂. This production of PGI₂ is stimulated by incubating endothelial cells with PGG₂ or with its precursor, arachidonic acid. Furthermore, PGI₂ synthesis is powerfully stimulated by cell-free plasma.

Pig aortic endothelial cells, aortic medial smooth muscle cells, and aortic adventitial fibroblasts, were isolated and grown in homogeneous monolayer cultures according to previously described methods^{7,8}. For experiments cells were detached (0.1% trypsin + 0.025% EDTA; 37 °C; 2 min) and suspended in serum-free HEPES-buffered Dulbecco's medium. Subsamples were resuspended in small volumes of calcium and magnesium-free phosphate-buffered saline (PBS) immediately before use. Platelet-rich plasma (PRP) was prepared by differential centrifugation of whole blood, anticoagulated with citrate or heparin; platelet aggregation was measured photometrically⁹ in 0.1 ml samples of human PRP¹⁰. All incubations were at 37 °C. Arachidonic acid (Sigma, Grade I) was prepared as the sodium salt, stored under nitrogen at –20 °C, and used within 2 weeks. Arachidonic acid was converted enzymatically¹¹ to 15-hydroperoxyarachidonic acid (15HPAA), a specific inhibitor of PGI₂ synthesis¹², and used immediately.

In previous studies of the effects of freshly isolated endothelium on platelets¹³ (and in preliminary studies with cultured vascular cells¹⁴) observations were first made by mixing cells with PRP, and we therefore performed similar experiments. Suspensions of 1.6–3.3 × 10⁴ endothelial cells, mixed with 0.1 ml human PRP, inhibited (with similar potency) platelet aggregation induced by ADP, collagen, vasopressin or the PGH₂ analogue (15S)-hydroxy-9α, 11α-epoxymethanoprostanoic acid (15E-dienoic acid (U44069)). This inhibitory effect was reduced by pre-incubating the endothelial cells (5 min) with 1 mM aspirin, an inhibitor of the cyclo-oxygenase component of the prostaglandin biosynthetic pathway¹⁵, and abolished by pre-incubating the cells with 1 mM 15HPAA. It was also reduced if PRP was pre-incubated (2 min) with 100 μM 2',5'-dideoxyadenosine (DDA), an inhibitor of platelet adenylate cyclase¹⁶. Thus, the inhibitory factor produced by mixing PRP with endothelial cells has properties characteristic of PGI₂.

In contrast, suspensions of either smooth muscle cells or fibroblasts, when mixed with PRP, induced platelet aggregation directly; the aggregation responses were similar to those produced by purified collagen, and were inhibited either by pre-incubating the vascular cells with collagenase or by pre-treating the PRP with aspirin or with a stimulant of adenylate cyclase (see Fig. 1). These results indicate that platelet aggregation was induced by cell-associated collagens known to be synthesised by these cells in culture^{17,18}. Because smooth muscle cells and fibroblasts aggregated platelets we could not, by direct mixing experiments, exclude the possibility that these cells might produce PGI₂.

Vascular tissue incubated with buffer alone, or with arachidonic acid or PGG₂, has been shown to produce sufficient PGI₂ for subsamples of the supernatant to inhibit platelet aggregation¹⁹.

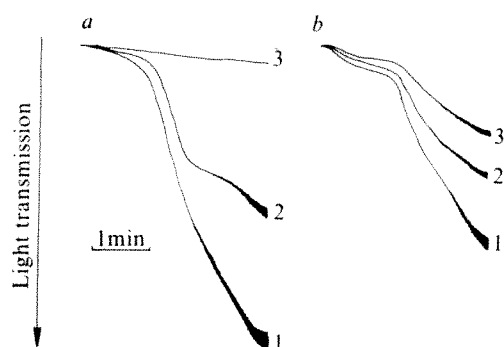


Fig. 1 Platelet aggregation induced by aortic smooth muscle cells or fibroblasts. *a*, Smooth muscle cells (5.2×10^4) in PBS were centrifuged (15,000g, 10 s) in an aggregometer cuvette and the supernatant was discarded. Human citrated PRP, 0.1 ml, was added, the mixture stirred at 37 °C and aggregation recorded. 1, Control response. 2, Cells pretreated with 0.2% collagenase in Dulbecco's medium (37 °C; 15 min). 3, PRP pretreated with 10 μM PGD₂ (37 °C; 2 min). *b*, Fibroblasts (6.6×10^3) prepared as above. 1, Control response. 2, Cells pretreated with collagenase. 3, PRP pretreated with 1 mM aspirin (37 °C; 5 min).

We therefore adopted this approach to characterise in more detail the localisation and regulation of PGI₂ production by vascular cells. To test for spontaneous production of PGI₂, cells were incubated in PBS for 2 min and subsamples of the supernatant added to PRP. To test for stimulation of PGI₂ production, cells were pre-incubated with 1 mM arachidonic acid or 0.2 μM PGG₂, washed in PBS alone for 2 min, and subsamples of these supernatants were tested as before. We have so far investigated PGI₂ production in aortic endothelial cell cultures, tested at between 2 and 17 passages in culture, derived from nine animals. Cells (5×10^3 – 10^5) cultured from five animals produced detectable levels of PGI₂ after incubation in PBS alone for 2 min, whereas incubations of up to 10^5 cells from the other four animals did not. We have also found PGI₂ production by endothelial cells cultured from the pig post-caval vein.

The inhibitor was characterised as PGI₂ by the following criteria: its activity was labile (half life ~ 5 min at 37 °C); its production was inhibited by pre-incubating endothelial cells with 15HPAA, aspirin, indomethacin, or mepacrine (an agent that blocks prostaglandin production in platelets by inhibiting

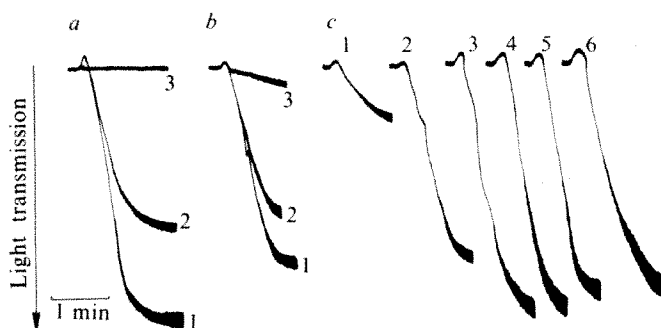
the plasmalemmal phospholipase A₂ responsible for endogenous arachidonic acid release²⁰); its effect was potentiated by pre-treating PRP with papaverine (a phosphodiesterase inhibitor) and inhibited by pretreating PRP with DDA (see Fig. 2). The inhibitor was active against primary aggregation induced by ADP, vasopressin and U44069; the effects of the latter two agonists are independent of ADP²¹, suggesting that in these conditions little, or none, of the inhibition is due to ADPase activity known to be present in aortic tissue^{22,23}. Secondary aggregation responses induced by ADP, U44069, collagen and arachidonic acid were also inhibited.

Cells from all animals tested produced easily detectable amounts of PGI₂ when stimulated with arachidonic acid or PGG₂. Although one culture, when retested after 16 passages, had lost the ability to produce detectable quantities of PGI₂, whether cells were stimulated with PGG₂ or added directly to PRP, other cells still produced PGI₂ after at least 17 passages. We have been unable to detect any enhanced PGI₂ production by pre-incubating endothelial cells (5 min) with 10–100 μg ml⁻¹ bradykinin or 10 μg ml⁻¹ angiotensin II, which stimulate production of prostaglandins E and F by cultured endothelium^{24,25}, and we have not observed any stimulation of PGI₂ production by thrombin at concentrations up to 25 U ml⁻¹. We have never observed the production of any inhibitor of platelet aggregation by smooth muscle cells or fibroblasts, even after stimulation with arachidonic acid or PGG₂.

Our results demonstrate that vascular endothelium, but not smooth muscle cells or fibroblasts, can synthesise PGI₂; this confirms and extends the preliminary observations made by Harker *et al.*¹⁴. Endothelial cultures from all animals tested, including those cells which failed to produce detectable PGI₂ on incubation with PBS alone, did inhibit platelet aggregation by producing PGI₂ when directly mixed with PRP. This implies that a factor (or factors) in PRP stimulate(s) PGI₂ production. One such factor could be a prostaglandin endoperoxide released from platelets; however, 5 min pre-incubation of PRP with 1 mM aspirin or 100 μM indomethacin had no effect on the inhibition by endothelial cells of primary platelet aggregation, which indicates that platelets are not necessary for the supply of precursor prostaglandin endoperoxides to endothelial cells for PGI₂ production. To investigate the possibility that other platelet-derived products might be responsible for stimulating PGI₂ production, we tested the effects on vascular cells of cell-free plasma, prepared in conditions which minimised secretion of platelet constituents. Incubation of 3.3×10^4 endothelial cells with 0.1 ml of cell-free plasma for 2 min, then centrifuging and resuspending in PBS alone for 2 min, resulted in the production of levels of PGI₂ (characterised by the criteria used above) detectable in as little as 2 μl of supernatant, and the amount of PGI₂ synthesised was directly related to the volume of cell-free plasma used as a stimulus (see Fig. 3). We have been able to stimulate PGI₂ production using plasma diluted as much as 50-fold with PBS. Smooth muscle cells (up to 3.3×10^5) did not produce PGI₂ on incubation with plasma. The stimulation of endothelial PGI₂ production was evident using human plasma prepared with citrate, heparin or EDTA, or human serum; and also with pig or rat plasma. PGI₂ production was stimulated equipotently by PRP or cell-free plasma, further indicating that in normal conditions platelet prostaglandin endoperoxides do not contribute significantly to endothelial PGI₂ production. The factor in cell-free plasma was heat labile (56 °C; 30 min), non-dialysable, and was still present after freezing of plasma.

The stimulation of PGI₂ production obtained using human cell-free plasma (which contains about 10 μM arachidonic acid²⁶) was comparable to that obtained by adding 500 μM arachidonic acid in PBS, even in the absence of albumin. It is therefore unlikely that PGI₂ synthesis is stimulated by precursor arachidonic acid carried on circulating plasma albumin. A more probable explanation is that the plasma factor stimulates PGI₂ production in a manner analogous to that by which RCS-RF (rabbit aorta contracting substance-releasing factor) stimulates

Fig. 2 Characterisation of PGI₂ production by endothelial cells. Suspensions of cells in PBS were incubated for 2 min at 37 °C centrifuged (15,000g, 10 s) and subsamples of the supernatant (50 μl, or 50 μl of PBS alone for controls) added to 50 μl human citrated PRP. After mixing for 1 min at 37 °C, aggregation responses to 1 μM ADP were measured. *a*, 1, Control; 2, supernatant from 1.6×10^4 cells; 3, supernatant from 6.6×10^4 cells. *b*, 1, Supernatant from 6.6×10^3 cells; 2, control, but PRP pre-incubated (37 °C, 2 min) with 20 μM papaverine; 3, as 1 but PRP pre-incubated with papaverine. *c*, 1, Supernatant from 3.3×10^4 cells; 2–6, as 1, but: 2, PRP pre-incubated (37 °C, 2 min) with 100 μM DDA; 3, cells pre-incubated (37 °C, 5 min) with 1 mM 15HPAA in PBS (before incubating in PBS alone); 4, cells pre-incubated (37 °C, 5 min) with 1 mM aspirin; 5, cells pre-incubated (37 °C, 5 min) with 100 μM indomethacin; 6, cells pre-incubated (37 °C, 5 min) with 50 μM mepacrine.



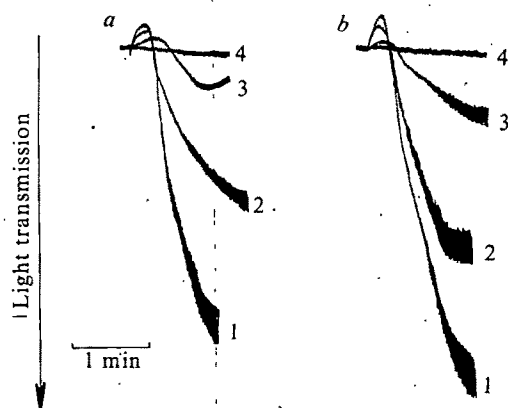


Fig. 3 Stimulation of endothelial cell PGI_2 production by cell-free plasma. Suspensions of 3.3×10^4 cells were pre-incubated in 0.1 ml human citrated cell-free plasma, or plasma diluted with PBS, for 2 min (37°C); centrifuged ($15,000g$, 10s), re-suspended in 0.1 ml PBS and incubated for a further 2 min. After centrifuging, subsamples of the supernatant were added to 50 μl of human citrated PRP, with sufficient PBS to give a final volume of 0.1 ml. After mixing for 1 min at 37°C , aggregation responses to 1 μM ADP were measured. *a*, Cells treated with undiluted plasma. 1, Control, 50 μl PBS alone; 2, 2 μl supernatant; 3, 10 μl supernatant; 4, 25 μl supernatant. *b*, 1, control; 2, 50 μl supernatant, but cells pretreated with 0.1 ml plasma diluted 1:10 with PBS; 3, as (2) but plasma diluted 1:1; 4, as (2) but plasma undiluted.

thromboxane A_2 production in lung tissue²⁷. This suggestion is strengthened by our finding that 5 min pre-incubation of endothelial cells with $4 \mu\text{g ml}^{-1}$ betamethasone, which antagonises RCS-RF activity²⁷, blocked PGI_2 synthesis. The lack of detectable effects of bradykinin and angiotensin II on PGI_2 synthesis, in contrast to their stimulation of endothelial PGE and PGF production^{24,25}, suggests that the plasma factor may selectively stimulate PGI_2 production.

The previous proposal that vascular PGI_2 production may be an important regulatory mechanism in haemostasis and thrombosis^{1,5} is reinforced by our findings that PGI_2 synthesis is localised in endothelial cells and that cell-free plasma alone stimulates PGI_2 production.

We thank the MRC for financial support. DDA was donated by Dr R. J. Haslam, PGG_2 by Dr E. W. Salzman, and all other prostaglandins by Dr J. E. Pike (Upjohn).

Note added in proof: Since this manuscript was submitted, three directly relevant papers have been published. Weksler *et al.*²⁸ present chemical evidence for prostacyclin production by cultured human and bovine aortic endothelium, but not by human fibroblasts. Baenziger *et al.*²⁹ report the synthesis of prostacyclin by cultured human aortic smooth muscle cells and fibroblasts. We therefore carried out further experiments, and by using $>3.3 \times 10^5$ smooth muscle cells, have found some strains to produce a transferable inhibitor of platelet aggregation. To produce comparable inhibition, however, required approximately $100 \times$ smooth muscle cells as endothelial cells. Moncada *et al.*³⁰ have shown that the conversion of PGH_2 to prostacyclin by rabbit aorta is at least tenfold greater (on a wet weight basis) in the intimal layer than in the media or adventitia.

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Received 3 November; accepted 2 December 1977.

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Sister chromatid exchange as an indicator of mutagenesis

SISTER chromatid exchange (SCE), that is, the reciprocal interchange of DNA between chromatids, is easily visualised in metaphase chromosomes^{1,2} and has been applied to study chromosome structure^{3,4}, chromosome damage⁵, and instability and DNA repair deficiency syndromes^{6–9}. Since SCEs can be induced by subtoxic doses of carcinogens and mutagens^{5,10–13}, their analysis offers the possibility of a rapid, sensitive and quantitative assay for genetic damage. We have begun to examine the relation between SCEs and mutations in Chinese hamster ovary (CHO) cells by quantifying the induction of SCEs in parallel with the induction of mutations producing 8-azaguanine resistance, that is, mutations predominately at the hypoxanthine phosphoribosyltransferase, *hprt*, locus. Since the conversion of a chemically induced DNA lesion to a SCE or mutation may depend on the nature of that lesion, we tested four chemicals that differ in their interaction with DNA—ethyl methanesulphonate (EMS; O^6 : N^7 guanyl alkylation ratio¹⁴ of 0.03), *N*-ethyl-*N*-nitrosourea (ENU; O^6 : N^7 guanyl alkylation ratio¹⁵ of about 0.35), the crosslinking agent mitomycin C (MMC)¹⁶ and the intercalator proflavine sulphate (PRO)¹⁷. Our results indicate a linear relation between induced SCEs and mutations. The relative efficiency, however, is different for each chemical.

As shown in Fig. 1, EMS, ENU and MMC produced a linear increase in both induced SCEs and mutations as a function of dose. All six regression lines for these chemicals had slopes significantly different from zero. Proflavine did not produce a significant increase ($P>0.05$) in either SCEs or mutations over the dose range tested, even though the highest dose (1.6 μM) reduced cell survival to approximately 60%. (Note the range of the ordinate in Fig. 1d compared to the other chemicals). For comparison with the other chemicals, however, we have treated these data as an indication of a positive response in both assays. Each of the four

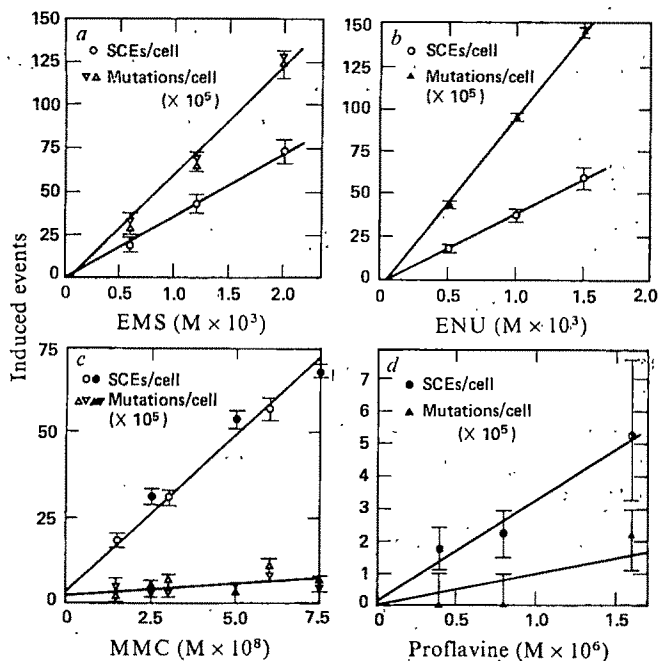


Fig. 1 The induction of SCEs and mutations as a function of dose for: a, EMS; b, ENU; c, MMC; and d, PRO. Circles represent SCEs per cell; triangles represent mutations per cell $\times 10^5$. A suspension-adapted clone of the CHO line designated SC1 was cultured in 200-ml spinner flasks in α minimum essential medium (MEM) with 10% foetal calf serum. With the cells growing exponentially at a density of $1.3 \times 10^5 \text{ ml}^{-1}$, 5-bromodeoxyuridine (BrdU), at a final concentration of $10 \mu\text{M}$, and the test chemicals were added to each culture. Control cultures contained only BrdU. After 18 h exposure (approximately 1.5 generations), the cells were collected by centrifugation, then rinsed once and resuspended in fresh medium. At each dose the culture was divided into two portions. The first fraction of each treated culture was subdivided into four identical 20-ml roller-tube cultures containing $10 \mu\text{M}$ BrdU, which were used to prepare chromosomes for SCE analysis. To ensure collection of the majority of second-division cells, sequential cell collection of the roller tubes was performed at 4-h intervals from 18 to 34 h. Colcemid (10^{-7} M final concentration) was added 4 h before cell collection. SCEs were differentiated by the FPG technique² and 25 cells were scored at each dose. For each 4-h collection interval an average SCE frequency for each dose was calculated by weighting the SCE frequency by the mitotic index of second-division cells in that interval. All operations before cell fixation or plating for mutations were performed in the dark or under a safelight. The second fraction of each treated culture was grown further, with low-ratio dilutions, in the absence of BrdU for mutation expression and selection. On days 4 and 5 (EMS and MMC) or day 4 (ENU and PRO) after removal of the chemicals these cultures were rinsed with serum-free medium and plated into regular medium (α -MEM + 10% dialysed foetal calf serum) for plating efficiency determinations, and into the same medium containing $15 \mu\text{g ml}^{-1}$ 8-azaguanine for selection of drug-resistant mutant colonies. The use of dialysed serum produced a reproducible assay in which all colonies isolated and tested were more drug-resistant than wild type¹⁸. The frequency of mutant colonies detected was proportional to cell inoculum in the conditions used, showing that cell density was not a complicating factor. Ten replicate 100-mm Petri dishes were seeded with 4×10^6 cells each and stained after 14 d of incubation. Plating efficiencies were determined by inoculating 300 cells per dish (five replicates) and staining after 8 d. Mutation expression was found to be maximal by four generations after exposure. The average mutation frequency in the presence of BrdU alone (controls) was $5.18 \pm 0.65 \times 10^{-5}$ (\pm s.e.m.) mutants per survivor. There was no apparent increase in the spontaneous mutation frequency from the presence of BrdU. The frequency of SCE in the controls was 7.03 ± 0.24 per cell averaged over five experiments. Control values were subtracted to obtain induced frequencies. The data were fit using a least squares linear regression with 0,0 as a data point. The open and closed triangles for MMC in (c) indicate two independent experiments. The two triangles for each dose in (a) and (c) represent day 4 and 5 platings; data were pooled for the regression analysis. Standard errors of the mean for the SCE data represent the sample time variation for each dose. Standard errors for the mutation frequencies represent Poisson counting error.

chemicals induced both SCEs and mutations over the same concentration range, suggesting a similarity in the sensitivity of the two assays. The slope of each regression line from Fig. 1 was used as a measure of the induction efficiency for SCEs or mutations, as shown in Table 1. On a molarity basis, both SCEs and mutations increase over four orders of magnitude as follows: $\text{EMS} < \text{ENU} < \text{PRO} < \text{MMC}$.

In Fig. 2 the frequency of induced SCEs per cell is compared with the frequency of induced mutations. The data show a linear relation between SCEs and mutations, but the ratio of induced SCEs to induced mutations (regression line slope) is different for each chemical (Table 1). Of the four chemicals, ENU is the least efficient inducer of SCEs compared with mutations (lowest ratio) while MMC is most efficient (highest ratio); these compounds differ by a factor of 15. Thus, it is clear that there is a positive, linear correlation between SCEs and mutations, but the relative efficiency of SCE and mutation induction varies with the compound. The type of lesion produced by each chemical and the nature of its repair probably determine the relative frequency of induced SCEs and mutations. Therefore, in order for SCEs to be a quantitative predictor of mutation induction, calibration curves similar to those in Fig. 2 may have to be derived for each agent.

Obviously many more chemicals must be compared before any general conclusions can be drawn concerning the relation of SCEs and mutations. Also, other genetic markers must be tested since azaguanine resistance may not have typical mutagen sensitivity for each chemical. If we do assume that this marker yields a representative mutation rate and that the ratio of SCEs to mutations (Table 1) is similar in human cells, then for the approximately 50,000 structural genes in man¹⁹, the above data suggest that for every SCE per cell: EMS produces 0.85 mutations per cell; ENU produces 1.2 mutations; PRO produces 0.28 mutations; and MMC produces 0.08 mutations. The use of this rapid cytological SCE analysis as a quantitative indicator of mutagenesis seems feasible but must be approached with caution since each chemical or class of compounds may respond differently. The SCE:mutation ratio may also differ with species, tissue type, dose rate, or other factors, making it necessary to establish the relationship for the exposure condition of interest. If an SCE:mutation ratio obtained *in vitro* can be extrapolated to cells *in vivo*, it will then be possible to predict mutation rates in man from SCE

Fig. 2 The relationship between induced SCEs and induced mutations. Each set of data was fitted by a least squares linear regression with 0,0 as a data point. For MMC, the two sets of solid triangles represent separate dose responses performed on different days; the two sets were combined to generate a single regression line.

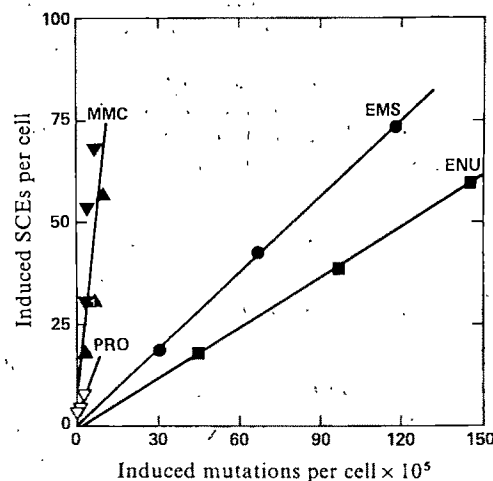


Table 1 Efficiency of induction of SCEs and mutation to azaguanine-resistance

Chemical	SCE per cell per mol per l		mutations per cell per mol per l		SCE per mutation	
	Absolute* ($\times 10^{-6}$)	Relative	Absolute* ($\times 1$)	Relative	Absolute† ($\times 10^{-4}$)	Relative
EMS	0.037	1.0	0.62	1.0	5.9	1.0
ENU	0.040	1.1	0.97	1.56	4.1	0.70
PRO	3.2	86	14.1	22.7	18	3
MMC	918	2.5×10^4	914	1.5×10^3	60	10

*Absolute efficiency is the slope of each dose response regression line in Fig. 1.

†Absolute ratio taken from slope of the regression lines in Fig. 2.

data derived from human cells exposed *in vivo* or *in vitro*.

We thank Dolores Piluso for technical assistance. This study was supported by US ERDA contract W-7405-ENG-48.

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Eosinophil chemotactic factor release from neutrophils by *Nippostrongylus brasiliensis* larvae

INFESTATIONS with metazoan parasites are the most efficient stimulus known for the induction of eosinophilia, and eosinophils have been shown to have an essential role in the elimination of worms^{1,2}. Eosinophil chemotactic factors have been derived from lymphocytes³⁻⁵, mast cells⁶ and the complement system⁷. We reported recently the *in vitro* release of a low molecular weight eosinophil chemotactic factor, ECF, from human neutrophils (PMN) during phagocytosis of zymosan⁸. The evidence presented here shows that neutrophils, after incubation with larvae of *Nippostrongylus brasiliensis* (N.B.), can also generate this same factor which selectively attracts eosinophils *in vitro*. As neutrophils are the first cells to accumulate at tissue sites where parasites are located⁹⁻¹¹, the attraction of eosinophils may present an early line of defence against the invading organism.

N. brasiliensis-infested Wistar rats^{12,13} were used as a model system as it is difficult to obtain sufficient numbers of organisms from infected humans. Larvae were obtained from the faeces of infested Wistar rats, washed twice in a Tris-albumin buffer containing calcium and magnesium¹⁴, and incubated with 1×10^7 purified human PMN in a total of 1 ml at 37 °C for the time indicated. PMN were separated from other peripheral leukocytes by Ficoll-Hypaque¹⁵ flotation, followed by dextran sedimentation to eliminate erythrocytes. Cells and larvae were removed by rapid centrifugation in the cold and the supernatants analysed for eosinophil chemotactic activity using the method described previously¹⁴. Results are expressed as the

number of eosinophils that have migrated completely through nitrocellulose filters (Sartorius) in five 10×40 microscopic fields. Human and guinea pig eosinophils used in these studies gave comparable results. Controls with buffer alone were always negative. This assay of chemotaxis does not measure the weak eosinophil chemotactic activity induced by very low histamine concentration¹⁴.

Table 1 shows the release of ECF with varying numbers of larvae after a 20-min incubation. Neither larvae nor cells alone induced eosinophil migration, but the mixture of both caused a dose-dependent release of eosinophil chemotactic activity. Similar results were obtained with normal human PMN, with normal rat peritoneal cells or with cells from rats infected with larvae 10 days earlier. The slight increase in histamine release (Table 1) was not detectable in other experiments, indicating that the PMN and not the mast cells were the source of the ECF. Similarly, histamine levels were not raised in the PMN supernatants, excluding the rather unlikely possibility of release of ECF from unsensitized basophils. Chromatography of either active cell supernatants on a Sephadex G-25 column showed that, in each case, the eosinophil chemotactic activity was eluted in the same fractions as the ECF obtained from basophils by anti-IgE (ref. 14) and from PMN on exposure to zymosan (molecular weight about 500) (ref. 16). The differently induced ECF preparations did not differ in their biological behaviour such as the preferential attraction of eosinophils¹⁶.

The time course of ECF-release from PMN was also studied (Table 2). After a lag period of 5 min, there was rapid release of the mediator for up to 20 min and then a gradual decline. A very similar curve can be obtained during ECF release from PMN with zymosan¹⁶. The decreasing activity after extended incubation has been shown to be due to an inactivator released together with ECF from PMN¹⁷. The lysosomal enzyme β -glucuronidase (measured by the method of Brittinger¹⁸) was released in a time dependent fashion with no decline in release rate at later time intervals.

Opsonisation of the larvae enhanced release of both ECF and β -glucuronidase from the cells. The totally passive role of the larvae in these processes was underlined by the fact that imotile parasites which had been stored in the frozen state for several weeks were just as efficient in stimulating the cells as

Table 1 Release of ECF and histamine from human PMN and rat peritoneal cells

Larvae (ml ⁻¹)	No. cells Eos/5 HPF	PMN 1×10^7 /ml		Rat peritoneal cells 1×10^7 /ml	
		Eos/5 HPF	% Histamine	Eos/5 HPF	% Histamine
0	0±0	0-0	1.0	0±0	2.0
8	0±0	0±0	1.0	15±2	3.0
80	0±0	32±4	0.5	41±4	5.0
800	0±0	109±7	1.0	279±12	6.0

ECF release was measured after 20 min incubation at 37 °C. Eos/5 HPF, eosinophils that have migrated through the filter in five high-power fields. Human PMN, 3% eosinophils, 6% lymphocytes, 0.2% basophils, 91% neutrophils. Rat peritoneal cells, 5% mast cells, 13% eosinophils, 7% neutrophils, 75% lymphocytes and macrophages.

Table 2 Release of ECF (Eos/5 HPF) and β -glucuronidase¹⁸ from 1×10^7 purified human PMN (> 90% neutrophils) incubated with 80 larvae in 1 ml buffer

Time (min)	ECF (Eos/5 HPF)	β -Glucuronidase (A_{540})
0	0 \pm 0	0.017
5	0 \pm 0	0.025
10	30 \pm 3	0.067
15	38 \pm 4	0.077
20	41 \pm 3	0.078
30	24 \pm 2	0.078
60	20 \pm 2	0.099

ECF and β -glucuronidase release was measured after incubation at 37 °C for the times shown.

fresh larvae.

The interaction of larvae and neutrophils was followed under the light microscope. During the first 5 min the larvae were seen to be moving about actively, and only an occasional cell was seen attached to them. By 10 min, the PMN had formed larger clumps which were partly trapping the larvae and the larvae were beating about actively with their free ends, apparently trying to free themselves from the cells. By 20 min and later, most larvae were completely encased and immobilised by the cells. At times, five or six larvae were enclosed by one cell clump. Very few larvae were completely unaffected by the process of entrapment.

In the tissue the interaction between invading organism and the PMN probably occurs only in a restricted fashion. But, since neutrophils are the most numerous scavengers of the body and since they do not have to be sensitised to function, they must be considered the first line of defence against parasites. Within a brief time, they then secrete a substance that attracts eosinophils to the site. Compared to neutrophils, these cells contain larger amounts of certain enzymes (histaminase¹⁹, arylsulphatase²⁰, and myeloperoxidase²¹) and of basic proteins²² which may enable them to modulate the local inflammatory event in a way more favourable to the host.

It is important to remember that the release of ECF from the PMN is a very specific process with regard to the stimulus—phagocytosis of certain bacteria, has no such effect (data not shown). In addition, inhibitory factors in cells¹⁷ and tissues²³ may modulate the influx of eosinophils to inflammatory sites, and the final outcome of the neutrophil-associated inflammatory process may depend on the complex interaction of several factors.

In the light of these results, the induction of eosinophilia during parasitic infestations may be viewed as follows. First, PMN accumulate close to the parasite and, during the process of phagocytosis, release ECF which selectively attracts eosinophils and also some neutrophils. Local activation of complement and production of the neutrophil and eosinophil chemotactic fragment C5a (ref. 7), will also play a part early on in sustaining the influx of both cell types. This latter line of defence may be continuously active during the subsequent course, especially when circulating immune complexes²⁴ become available as an additional means of complement activation. Finally, lymphocyte- and mast cell-dependent mechanisms of induction of eosinotaxis are activated after sensitisation and production of specific IgE has occurred^{25,26}. It may well be that the response produced at this time is more effective in evoking and sustaining eosinophilia and in eliminating the invading organism. But the mechanisms induced at earlier times may serve to abort very mild infestations and/or to set the stage for a better immune response and a favourable outcome for the host.

This work was supported by the Deutsche Forschungsgemeinschaft.

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Received 6 October; accepted 9 December 1977.

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Novel type of murine B-cell lymphoma

ALTHOUGH several murine T-cell lymphomas have been well characterised (reviewed in ref. 1), only a single group of murine B-cell lymphomas, the Abelson lymphomas, has been described². The Abelson lymphoma arose in a steroid-treated BALB/cCr mouse inoculated with Moloney leukaemia virus², expresses surface immunoglobulin^{3–4} and can be transmitted by an infectious murine leukaemia virus². We report here a new IgM-bearing B-cell lymphoma distinct from the Abelson lymphoma in its induction, pathogenesis and antigenic characteristics and which has many features in common with the widely studied guinea pig L₂C lymphoma^{5–6}.

The phenomenon reported here was observed during the selection of a new strain of mice—B10-H-2^a H-4^bp/Wts⁷. This 'double congenic' strain differs from the background strain C57BL/10 (H-2^b, H-4^a) at two segments of its genome; one surrounding the H-2 locus and another surrounding the H-4 locus. When B10-H-2^a H-4^bp/Wts mice were immunised with sheep erythrocytes and their spleen cells or sera were transferred to syngeneic or F₁ hosts (protocol previously described⁸), a lymphomatous disease resulted. Symptoms suggesting a lymphomatous disease occurred in more than 50% of the cell and serum recipients within 150 d of transfer. Strains congenic to B10-H-2^a H-4^bp/Wts at the H-4 (B10.A; H-2^a H-4^a) and H-2 (B10.129(21M); H-2^b, H-4^b) loci did not develop lymphoma when treated in the same manner.

The disease is characterised by hypersplenomegaly, lymph node enlargement and whitish nodes in the liver. The primary disease process does not seem to involve the thymus or bone marrow and the serum from tumour-bearing animals does not seem abnormal on electrophoresis. Several of these primary tumour-bearing animals have been observed and one B10-H-2^a H-4^bp/Wts tumour was selected for transplantation and characterisation.

The cell surface antigens expressed by the lymphoma cells are presented in Tables 1 and 2. When these transplanted ascites tumour cells were assayed for surface immunoglobulin with a polyvalent rabbit anti-mouse immunoglobulin, more than 80% of them were consistently found to be positive for Ig by indirect immunofluorescence (Table 1). Studies with heavy-chain-specific reagents demonstrated that most if not all of these Ig+ cells specifically expressed IgM, and not IgA or IgG. Qualitatively, the large lymphoblast-like tumour cells demonstrated very bright diffuse fluorescence indicating a high

surface concentration of IgM. Attempts to kill the lymphoma cells with rabbit anti-mouse immunoglobulin and complement were unsuccessful; yet, this antiserum induces strong agglutination of tumour cells (unpublished observation).

That this murine lymphoma does not bear thymus cell antigens is conclusively demonstrated by the results presented in Table 2. Although the rabbit anti-mouse brain antiserum used to detect mouse T cells reacts with most of the tumour cells by indirect immunofluorescence, this phenomenon does not depend on a T-cell-specific antigen. Absorption of the rabbit anti-mouse brain antiserum with tumour cells does not remove the antibodies directed at normal mouse thymocytes. Additionally, absorption of the rabbit anti-mouse brain with thymocytes does not remove its reactivity with tumour cells; yet, does remove the anti-T-cell antibodies. The nature of this cross-reactive antigen is being investigated. Negative results from dye exclusion cytotoxicity assays with a specific anti-Thy 1.2 antiserum, as well as the rabbit anti-mouse brain, further substantiate that this tumour is not of thymic origin (Table 2).

Our preliminary evidence clearly distinguishes the B10-H-2^a H-4^bp/Wts B lymphoma from the only other characterised group of murine B-cell lymphomas, the Abelson lymphomas. There are several important differences. First, induction: the lymphoma reported here is induced in recipients of sera or cells from immunologically stimulated B10-H-2^a H-4^bp/Wts donors. Cell transfers from other congenic strains do not induce tumorigenesis. Conversely, Abelson lymphoma was induced in a BALB/cCr mouse treated with an immunosuppressant and inoculated with Moloney leukaemia virus². Second, pathogenesis: the B10-H-2^a H-4^bp/Wts lymphoma mainly involves the peripheral lymphoid organs, in particular the spleen. This contrasts with the Abelson lymphoma which primarily involves the bone marrow cells and the meninges¹¹. Third, antigenic characteristics: although both Abelson B-cell lymphoma and the lymphoma reported here bear surface immunoglobulin, at least their quantity of expression differs. Sklar *et al.*³ have reported that cells infected with Abelson virus express surface immunoglobulin; yet, surface immunoglobulin can only be detected by very sensitive detection methods⁴ and only on a minor percentage of these tumour cells³. This is not the case with the B10-H-2^a H-4^bp/Wts lymphoma. Ninety per cent of these cells show very bright fluorescence with an anti- μ specific antiserum (Table 1). These lymphoid cells are probably monoclonal, and the nature of the idiotypic determinants of this surface IgM is being investigated.

Table 1 Expression of surface immunoglobulin by B10-H-2^a H-4^bp/Wts lymphoma cells

Primary antisera	% Immunofluorescence		
	Spleen	Thymus	Tumour
Rabbit anti-mouse Ig	35	<1	83
Goat anti- μ *	45	<1	90
Goat anti- α *	<1	<1	<1
Goat anti- γ *	10	<1	<1

Indirect immunofluorescence was performed as follows. 5×10^6 cells were suspended in 0.1 ml of the appropriate primary antiserum and incubated for 30 min at 4 °C. Cells were washed extensively in cold phosphate-buffered saline (PBS) containing 0.1 M Na₂SO₃. Cells were then resuspended in 0.1 ml of the appropriate fluorescein-conjugated secondary antiserum and incubated for 30 min at 4 °C. All cells were washed extensively in cold PBS-0.1 M Na₂SO₃, resuspended in a small volume of PBS-0.1 M Na₂SO₃ containing 50% glycerol, and observed under a Zeiss microscope equipped for fluorescent microscopy. Results are expressed as percentage of cells positive for fluorescence/total cells counted. Controls for phagocytosis and adherence of Ig to Fc receptors consisted of spleen, thymus and tumour cells incubated in the second antiserum only. Less than 1% of the control cells were positive. All antisera were diluted in PBS-0.1 M Na₂SO₃ to prevent capping. Rabbit anti-mouse Ig was prepared by standard methods and was used at a 1:30 dilution. The fluorescein-conjugated goat anti-rabbit Ig (Meloy, Springfield, Virginia) was diluted 1:40.

*Heavy-chain-specific antisera prepared in goats (Meloy) were diluted 1:20 in PBS-0.1 M Na₂SO₃. The second antisera, fluorescein-conjugated rabbit anti-goat Ig (Meloy) were diluted 1:40 before use.

Table 2 Failure to detect T-lymphocyte antigens on the B10-H-2^a H-4^bp/Wts murine lymphoma

Antisera	% Immunofluorescence			% Cytotoxicity		
	Spleen	Thymus	Tumour	Spleen	Thymus	Tumour
anti-Thy. 1.2	ND	ND	ND	30	100	<1
Rabbit anti-mouse brain*	34	100	90	35	100	<1
Rabbit anti-mouse brain absorbed with thymocytes†	ND	<1	88	<5	<1	ND
Rabbit anti-mouse brain absorbed with tumour cells‡	ND	100	<10	15	90	ND

Indirect immunofluorescence was performed as described in Table 1. Controls for phagocytosis and adherence of Ig to Fc receptors showed that less than 1% of the cells were positive. Dye exclusion cytotoxicity was performed using a minor modification of the technique described by Frelinger *et al.*⁹. Guinea pig complement (Gibco) was the source of complement at a dilution of 1:8. Controls, consisting of cells with antiserum or complement alone, were consistently negative. NIH Contract anti-Thy 1.2 (AKR anti-C3H) antiserum was provided by the Research Resources Branch of NIH. This antiserum killed 100% of the control normal thymocytes at a dilution of 1:10. ND, not determined.

*Rabbit anti-mouse brain antiserum was produced in our laboratory by the technique of Golub¹⁰. For indirect immunofluorescence it was used at a dilution of 1:20 and for cytotoxicity at a dilution of 1:10.

†Rabbit anti-mouse brain antiserum (0.6 ml, 1:10 dilution) was absorbed twice with three normal thymus equivalents for 30 min at 25 °C and used at a dilution of 1:10.

‡Rabbit anti-mouse brain antiserum (0.6 ml, 1:10 dilution) was absorbed twice with 0.2 ml of packed tumour cells for 30 min at 25 °C and used at a dilution of 1:10.

The B10-H-2^a H-4^bp/Wts lymphoma is interesting from another viewpoint. Because only cells or sera from immunologically stimulated B10-H-2^a H-4^bp/Wts mice seem to be able to induce this disease, something involving the combination of the H-2^a and H-4^b chromosomal segments in association with an immune stimulus may make this phenomenon possible. Recently a second B-cell lymphoma, similarly induced in B10-H-2^a H-4^bp/Wts mice, has been established in transplantation and found to bear surface IgM with an idiotype distinct from that of the first tumour. Work is in progress to characterise these tumours and to define the genetic, virological and immunological aspects of these B-cell lymphomas. To add to the well known L₂C guinea pig leukaemia, these murine B-cell tumours provide another excellent model for a similar human malignancy, chronic lymphocytic leukaemia. The murine model has advantages over the guinea pig system in that large experimental groups can be used conveniently, comparisons can be made between different individual tumours and the genetics of the mouse have been much better defined.

We thank Drs James Folds and Marvin Tyan for antisera and Becky Owens for technical assistance.

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Received 1 September; accepted 16 November 1977.

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Induction of antigen-specific human suppressor T lymphocytes *in vitro*

INTENSIVE studies in a variety of animal species have led to the recognition that cell interaction is important for the generation of an immune response¹. The participation of macrophages and of T and B lymphocytes in the process of antibody formation has been demonstrated both *in vivo* and *in vitro*^{2,3}. More recently, attention has been focused on the mechanism(s) regulating the development and expression of immune reactivity, leading to the discovery in a number of animal species of subpopulations of T cells with different functions, such as helper cells (Th) and suppressor cells (Ts) (refs 4-6). Dosch and Gelfand⁷ recently reported that the induction of plaque forming cells (PFC) in human tonsillar or blood lymphocytes is abolished if a (relatively) high antigen dose is used and we have confirmed this finding using the antigens sheep red blood cells (SRBC), ovalbumin (OA) and haemocyanin (Hc)⁸. We have extended these studies and report here the *in vitro* generation of antigen-specific Ts cells in man.

Human peripheral blood lymphocytes (PBL), isolated by density centrifugation⁹ were cultured in tubes for 6 d with or without antigen (SRBC or OA). The cells were then collected, washed and transferred to monolayers of untreated SRBC or of OA-coated SRBC. Primed lymphocytes secreting specific antibody can be recognised by plaque formation. The specificity of this *in vitro* immune response has been firmly documented^{7,8}. The magnitude of the PFC response is antigen dose-dependent, as illustrated in Table 1. Optimal results in the conditions used were obtained at an antigen concentration of $3 \mu\text{g ml}^{-1}$ for OA and at a lymphocyte:SRBC ratio of 1:1. The low number of PFC found in cultures stimulated with a high dose of antigen suggested the generation of suppressor cell activity. However, in these conditions B cells might be inactivated by the high antigen concentration without interference of Ts. To differentiate between these two possibilities PBL were cultured for 24 h with a high dose of OA ($100 \mu\text{g ml}^{-1}$), the cells were collected, washed and added to an equal number of autologous PBL. The cell mixture was cultured in standard conditions (see Table 1) at optimal antigen concentration (OA $3 \mu\text{g ml}^{-1}$) for 6 d and the number of PFC determined. The degree of suppression in these cultures ranged from 62 to 94%, with an average of $80.7 \pm 14.5\%$ (mean \pm s.e.m.) in five different experiments (Table 2). These findings indicate that the low number of PFC generated in cultures

Table 1 Role of antigen concentrations in the PFC response of PBL

OA ($\mu\text{g ml}^{-1}$)	PFC per 10^6 lymphocytes	Lymphocyte:SRBC ratio	PFC per 10^6 lymphocytes
0.3	700(150-1,600)	10:1	299(1,805-560)
1.0	1317(920-2,000)	1:1	1538(1,026-2,100)
3	1418(600-3,000)		
30	230(25-800)	1:10	240(37-620)

For the induction of PFC to OA and SRBC, cultures were set up in 17×100 mm tissue culture tubes (Falcon) containing 5×10^6 PBL in 10 ml RPMI-1640 medium supplemented with 10% heat inactivated AB serum (of a pooled stock of three donors), penicillin (100 IU ml^{-1}), streptomycin ($100 \mu\text{g}$), Fungizone ($25 \mu\text{g ml}^{-1}$), L-glutamine (2 mM) and antigen at different concentrations. When SRBC were used as antigen, the AB serum was absorbed three times with SRBC to prevent pseudo-plaque formation¹⁰. The cultures were kept in a humidified atmosphere of 5% CO_2 in air. After culture for 6 d cells were collected, washed twice in RPMI-1640 at 4°C and assayed for PFC according to the method of Dosch and Gelfand⁷. The data shown are expressed as the arithmetical mean of values obtained in 10 individual experiments. Within each experiment determinations at the various antigen doses were done in six fold. Values in parentheses represent the ranges.

Table 2 Effect of *in vitro* induced suppressor cells on the anti-OA PFC response

Expt no.	Day 1 PBL*	Day 2 Suppressor cells†	Day 6 PFC per 10^6 lymphocytes	% Suppression†
1	10×10^6	—	1,810	
	5×10^6	5×10^6	245	86.5
2	10×10^6	—	737	
	5×10^6	5×10^6	233	68.3
3	10×10^6	—	857	
	5×10^6	5×10^6	48	94.3
4	10×10^6	—	1,236	
	5×10^6	5×10^6	91	92.6
5	10×10^6	—	5,357	
	5×10^6	5×10^6	2,020	62.3

*In control experiments 10^7 PBL were treated as outlined below except that the antigen dose used was OA $3 \mu\text{g ml}^{-1}$.

†In order to generate suppressor cells 10^7 lymphocytes were cultured in the presence of OA $100 \mu\text{g ml}^{-1}$ in 10 ml RPMI-1640 containing antibiotics L-glutamine and 10% AB serum for 24 h. Cells were collected, washed twice in RPMI-1640 and 5×10^6 of these cells were added to 5×10^6 PBL that had been primed with OA $3 \mu\text{g ml}^{-1}$ 24 h earlier and cultured for an additional period of 5 d.

‡The % suppression was calculated according to the formula:

$$100 - \frac{\text{No. PFC per } 10^6 \text{ lymphocytes in suppressed cultures}}{\text{No. PFC per } 10^6 \text{ lymphocytes in normal cultures}} \times 100$$

with high doses of OA is the result of a suppressive action of PBL induced by contact of these cells with OA in high concentrations. To identify the cell type responsible for the suppression of *in vitro* antibody response, PBL were depleted of adherent cells and separated into T and non-T cells (see legend to Table 3). The isolated T lymphocytes and the non-T cells (B and null cells) were supplemented with 5% adherent cells and incubated for 24 h with OA $100 \mu\text{g ml}^{-1}$. The cells of these two different populations were then washed and added to normal PBL primed with OA $3 \mu\text{g ml}^{-1}$ 24 h

Table 3 The nature of the *in vitro* induced suppressor cells

Cell mixtures added to PBL with antigen	Treatment	PFC response to OA (PFC per 10^6 lymphocytes)		
		Expt 1	Expt 2	Expt 3
T cells	—	906	686	616
Non-T cells*	—	—	—	—
T cells	100 μg OA	267(70.5)†	240(65)	185(70)
Non-T cells	—	—	—	—
T cells	—	715(21.1)	750(—9.3)	608(1.3)
Non-T cells	100 μg OA	—	—	—

PBL, isolated by density gradient centrifugation, were depleted of non-lymphoid mononuclear cells by incubation in Falcon flasks (45 min at 37°C) in RPMI-1640 supplemented with 20% foetal bovine serum (PBL_{acc}). The adherent cells (monocytes, macrophages) were recovered by gently scraping with a rubber policeman, washed twice in Ca^{2+} - and Mg^{2+} -free Earle's balanced salt solution supplemented with EDTA (0.02 mM) and resuspended in culture medium. The non-adherent cells were separated into T cells and non-T cells according to the method of Moretta *et al.*⁶. The isolated T cells and the non-T cells (B and null cells) were supplemented with 5% adherent cells and incubated for 24 h with OA $100 \mu\text{g ml}^{-1}$ or without antigen in culture medium (see Table 1). The cells were then washed twice with RPMI-1640. Since it has been found that the concentration of OA necessary for the induction of maximal numbers of PFC increases with the numbers of T cells present in a given cell suspension^{7,8} T cells and non-T cells, previously incubated with OA $100 \mu\text{g ml}^{-1}$ were reconstituted with normal non-T cells and T cells respectively to obtain cell mixtures with T:B ratios equally to those of normal PBL suspension. 5×10^6 Lymphocytes of these cell mixtures were added to 5×10^6 PBL_{acc} reconstituted with 5% macrophages and incubated with OA $3 \mu\text{g ml}^{-1}$ 24 h earlier. After 5 d PFC assay was performed⁷.

*T-cell contamination in the non-T cell population was 3.5%, < 0.5% and 1.5% respectively in Expts 1, 2 and 3.

†The values in parentheses represent the percentages of suppression calculated according to the formula in the legend to Table 2.

earlier (the suppressive effect in these conditions is optimal, data not shown). After 5 d of culture PFC assay was performed. The results (Table 3) show clearly that after incubation for 24 h with a high dose of OA, non-T cells are not able to suppress PFC response *in vitro*. In contrast, suppressive activity can be induced in identical conditions in a purified T-lymphocyte population. This might be interpreted as the result of activation or generation of Ts. It was realised that a high concentration of antigen, carried over by cells previously incubated with the antigen, might have induced B-cell unresponsiveness. There are, however, several arguments that counter this possibility. First, in contrast to T cells, non-T cells after incubation with OA $100\text{ }\mu\text{g ml}^{-1}$ failed to suppress the anti-OA PFC response. Therefore, neither B lymphocytes nor adherent cells (macrophages) are able to carry over sufficient amounts of antigen to inhibit PFC formation. Antigen carry-over by T cells is therefore highly unlikely. Second, Cobi Heynen *et al.* (in preparation) found evidence that a major subpopulation of T cells bearing a receptor for the Fc fragment of IgM ($\text{T}\mu$) is not able to suppress an anti-OA PFC response after treatment with high concentrations of this antigen. Taken together these results fail to support induction of B-cell unresponsiveness by the carry-over of high antigen concentrations as the predominant mechanism of the observed suppression.

Table 4 Specificity of suppression by PBL treated with OA

PBL* treated with ($100\text{ }\mu\text{g ml}^{-1}$)	Antigen in culture	PFC response against	% Suppression of PFC response†		
			Expt 1	Expt 2	Expt 3
OA	SRBC	SRBC	-8	26.7	10.8
OA	OA	OA	92	92.6	73.9

*Suppressor cells were generated as described in the legend to Table 2.

†Percentage suppression was calculated according to the formula in Table 2 legend.

The suppression obtained in our system is antigen specific, as Ts induced with OA did not suppress PFC formation against SRBC (Table 4). Also, Ts induced by incubation with high numbers of SRBC specifically suppress PFC response against SRBC but have no effect on OA-specific PFC (data not shown). It should be emphasised that the suppressive effect described here is on the IgM response. Since it has been found in mice that IgM responses are more difficult to suppress than secondary IgG formation^{11,12}, it would be interesting to study the effect of induced Ts on restimulated cultures. Attempts are currently being made to obtain an anamnestic immune response *in vitro*.

The possibility of inducing *in vitro* antigen specific suppressor cells might have far reaching implications in immune manipulation in man.

We thank Drs B. J. M. Zegors and F. Gmelig Meyling for useful discussions and Mrs Ineke Dollekamp for her technical assistance. This work was supported (in part) by the Foundation for Medical Research (FUNGO) which is subsidised by the Netherlands Organisation for the Advancement of pure research (ZWO).

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Received 9 November; accepted 9 December 1977.

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Glutamate neurotoxicity and Huntington's chorea

McGEER and McGeer¹ recently reported that an intrastriatal injection of glutamate results in biochemical changes in brain similar to those associated with Huntington's chorea. They postulated that glutamate, a putative excitatory transmitter and an 'excitotoxin' abundantly present in brain may have a role in the pathophysiology of Huntington's chorea. Their evidence for this postulate must be considered very preliminary, however, as the biochemical changes induced by a 50 nmol dose of glutamate were not striking and it was not determined whether these changes were accompanied by neuronal degeneration¹. We have injected various doses of glutamate directly into the rat striatum and examined the striatum for histopathological changes 21 d later. Here we report that doses of glutamate much higher than 50 nmol definitely cause striatal neurones to degenerate, whereas no neurotoxic reaction results from injecting equally high doses of control compounds such as NaCl or γ -aminobutyric acid (GABA).

Glutamate and its several neuroexcitatory analogues, when administered systemically to experimental animals, destroy neurones in certain brain regions which lack adequate blood brain barriers to these compounds². Certain analogues, such as kainic acid (KA), *N*-methyl aspartic acid (NMA) and homocysteic acid (HCA) are more powerful than glutamate in either excitatory or toxic activity^{2,3}. The introduction of tiny amounts of these potent analogues by microinjection directly into the rat brain destroys local neurones without damaging axons passing through or terminating in the region⁴; lesion size is directly proportional to the known excitatory potency of the injected compound, the order of potencies being KA>NMA>HCA. A single injection of KA (5-10 nmol) into rat striatum results in rapid loss of striatal neurones⁵⁻⁷ and specific changes in transmitter enzymes^{1,5} similar to those known to occur in Huntington's chorea. This suggests that the more potent analogues of glutamate may be useful tools for studying Huntington's chorea. The findings reported here are consistent with the related but potentially more important proposal¹ that glutamate itself might have a role in the loss of striatal neurones in Huntington's chorea.

Twenty-four adult male Wistar rats (Harlan) weighing 250 g were used in these experiments. Monosodium L-glutamate (Glu) and GABA were obtained from Sigma. All compounds were introduced stereotactically into the striatum as previously described⁷ through a 30 gauge cannula by the method of Swanson *et al.*⁸. Glutamate was dissolved in sterile H₂O in various concentrations to permit testing four doses (50, 150, 500 and 1,000 nmol) in a volume of 1 μ l. Aqueous solutions of two control compounds, NaCl and GABA were administered at 1,000 nmol to match the highest dose of glutamate used. The glutamate and GABA solutions were at pH 7.0 and NaCl was at pH 6.1. At least four animals were used to test each dose of each compound. Each animal, under pentothal anaesthesia, was given a single 1- μ l injection of solutions containing one of the three compounds. The solutions were injected slowly over a 5-min

period and the cannula was left in place for an additional 10 min before removal to prevent retrograde movement of the injected solution up the cannula tract. Animals were killed 21 d after injection by glutaraldehyde-paraformaldehyde perfusion fixation under chloralhydrate anaesthesia and 3/4-mm serial slabs collectively encompassing the entire striatum were additionally fixed in osmium tetroxide and processed for combined light and electron microscopic examination by methods previously described^{9,10}.

None of the striata injected with GABA or NaCl had detectable histopathological changes other than minor changes expected from cannula insertion. The injection site could be identified by a small scar, but this was surrounded on all sides by healthy looking neurones in normal numbers. The appearance of a GABA injection site is illustrated in Fig. 1. The failure of NaCl injection to induce a neurodegenerative reaction in rat brain is reported elsewhere²².

In the striata of animals which received 50 or 150 nmol of glutamate there were indications about the injection site of a more extreme tissue reaction than was induced by GABA or NaCl, but definitive evidence for neuronal loss was lacking. It should be noted, of course, that the deletion of a very small number of neurones would be difficult to demonstrate with any confidence. All animals which received 500 nmol of glutamate had a decidedly larger area of tissue disturbance which we judged to be evidence for a neurotoxic reaction, based on the abundant reactive cells present and of the reduced number of neuronal profiles detectable. We estimated that approximately 5–10% of the total striatal population of neurones was deleted by 500 nmol of glutamate. A much larger area of tissue disturbance was evident in all animals which had received an injection of 1,000 nmol of glutamate. Approximately 30% of the total striatal neuronal population was deleted by this dose of glutamate. There were numerous reactive cells distributed about the lesioned area and neuronal profiles were absent but axonal bundles remained intact (Fig. 2). Thus, the effect seemed to be the characteristic dendrosomatotoxic but axon-sparing type of neurodegenerative effect which excitotoxic compounds are known to produce^{4,5,7,10,11}.

Our observation that an intrastriatal injection of glutamate destroys striatal neurones is consistent with the finding of McGeer and McGeer¹ that injecting glutamate into the

Fig. 1 A light micrograph depicting a region of striatum where 1000 nmol of GABA was injected 21 d previously. A scar is detectable where the cannula tip penetrated the striatal tissue but healthy neurones in normal numbers surround the scar ($\times 280$).

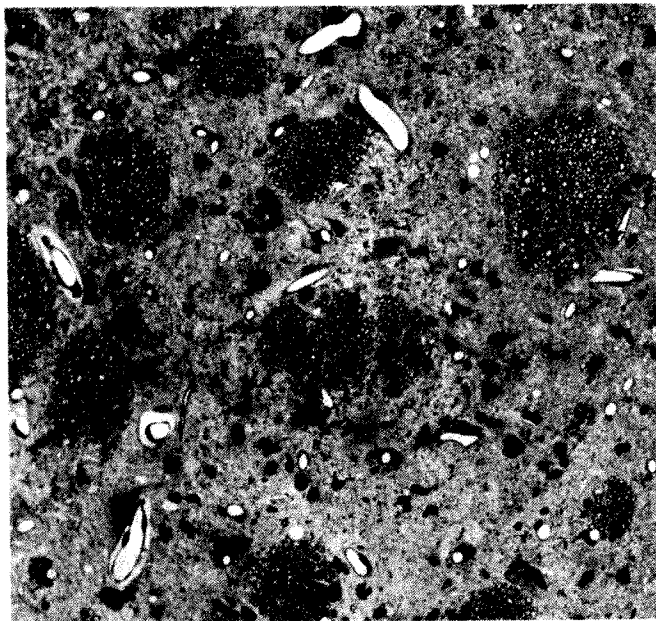
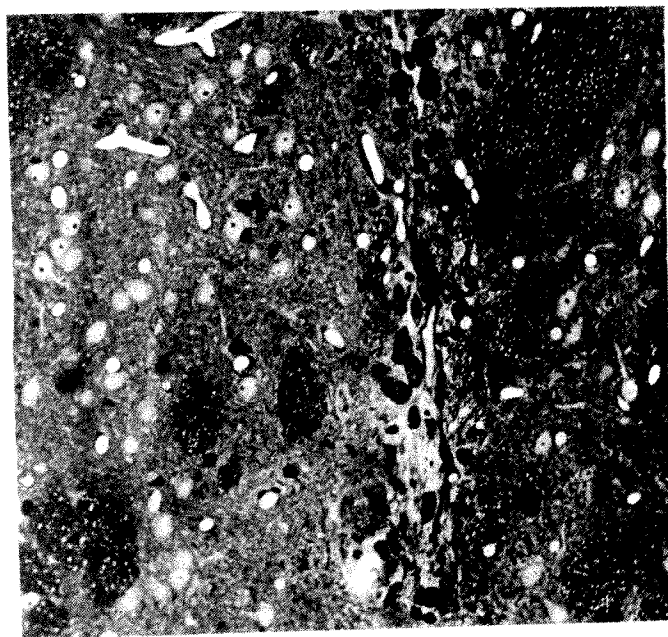


Fig. 2 A light micrograph depicting a region of striatum about 500 μ m away from the site where 1,000 nmol of Glu was injected 21 d previously. Although all intrinsic striatal neurones have degenerated beyond recognition, bundles of axons passing through the striatum remain intact and have a normal appearance ($\times 280$).

striatum causes a loss of the transmitter-synthesising enzymes, cholineacetyltransferase and glutamic decarboxylase which are considered markers for striatal neurones. It is puzzling, however, that we detected significant neuronal loss from doses of glutamate substantially higher than the 50 nmol which produced enzyme changes in the experiments of McGeer and McGeer. Presumably, a reduction in transmitter marker enzymes would occur only to the extent that neurones containing these enzymes are destroyed. Additional studies are needed to clarify whether such an expectation is warranted and to explain the apparent discrepancy between our findings and those of McGeer and McGeer¹.

Although our results suggest that glutamate is a weak neurotoxin compared to KA, we suspect this may be due in part to mechanisms in normal brain which function to protect neurones more effectively from glutamate than from KA. For example, the high affinity uptake system which, by removal of glutamate from excitatory receptor loci may control its excitotoxic potential, is relatively inaccessible to KA¹². This inactivation system may be efficient enough in normal striatum to prevent glutamate, unless injected in large amounts, from destroying local neurones. If, in the course of an individual's life, this vital homeostatic mechanism became dysfunctional, however, glutamate released at excitatory synapses might gradually accumulate in the synaptic cleft region in sufficient concentration to cause excitotoxic degeneration of glutamergically innervated neurones. In view of accumulating evidence^{13–17} suggesting that striatal neurones receive massive input from terminals utilising glutamate as transmitter, it is reasonable to propose¹ that glutamate may be involved in the degeneration of striatal neurones in Huntington's chorea. Indeed, for heuristic purposes, we would advance the more specific proposal that an adult-onset disturbance in the transport system for intracellular reuptake of glutamate might underly this neurodegenerative syndrome. This may be a testable hypothesis, depending on the technical feasibility of assaying the high affinity glutamate uptake capacity of striatal tissue obtained at autopsy from brains of patients with Huntington's chorea.

This work was supported in part by USPHS grants

DA-00259, NS-09156 and Research Career Development Award MH-38894 (J.W.O.).

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Received 28 September; accepted 5 December 1977.

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Morphine and β -endorphin inhibit release of noradrenaline from cerebral cortex but not of dopamine from rat striatum

β -ENDORPHIN is a large polypeptide containing 31 amino acid residues, the first five of which are identical with methionine-enkephalin¹ which has potent naloxone-sensitive analgesic properties² and opiate activity in receptor binding assays in guinea pig ileum^{3,4}. It has been shown that morphine depresses the potassium-induced release of ³H-noradrenaline in the rat cerebral cortex⁵ and of ³H-dopamine in the rat striatum⁶. These effects are blocked by the opiate antagonist naloxone and are probably due to the activation of presynaptic inhibitory opiate receptors located in noradrenergic and dopaminergic nerve endings of the rat brain. While met-enkephalin reproduced the effects of morphine in rat cortex slices⁷, it failed to reduce the release of dopamine from the rat striatum⁶. In view of these results, it was considered of interest to examine under similar experimental conditions, the effects of morphine and of β -endorphin on the potassium-stimulated release of ³H-dopamine from striatal slices and of ³H-noradrenaline from cerebral cortex slices of the rat⁸. In contrast to Loh *et al.*⁶, who more recently have not been able to reproduce their data (E. L. Way, personal communication), we find no evidence for the inhibition of the potassium-stimulated overflow of ³H-dopamine by β -endorphin and morphine in the striatal slices of the rat.

Male rats of 150 to 200 g were killed by decapitation. Slices of 0.3 mm thickness were obtained from the rat corpus striatum and occipital cerebral cortex with a McIlwain tissue chopper. The slices were transferred to the incubation medium in an open lucite cylinder with a nylon mesh fitted to the bottom as a small basket⁹. The whole system was placed in a 30-ml beaker with 5 ml of Tyrode solution containing 61 μ M ascorbic acid and 69 μ M EDTA equilibrated with a 5% CO₂ in O₂ and maintained at 37 °C.

Striatal and occipital cortex slices were incubated for 30 min with 2 μ M ³H-dopamine and 0.9 μ M (\pm) ³H-noradrenaline, respectively. The tissue was washed by transferring to successive beakers, and the spontaneous outflow of radioactivity into the medium was followed for 65 min before the release of the labelled transmitter was elicited by 1 min exposure to 20 mM K⁺. Two periods of stimulation with K⁺ were applied in each experiment, S₁ and S₂. The interval between S₁ and S₂ was 40 min. The overflow of the labelled transmitter elicited by

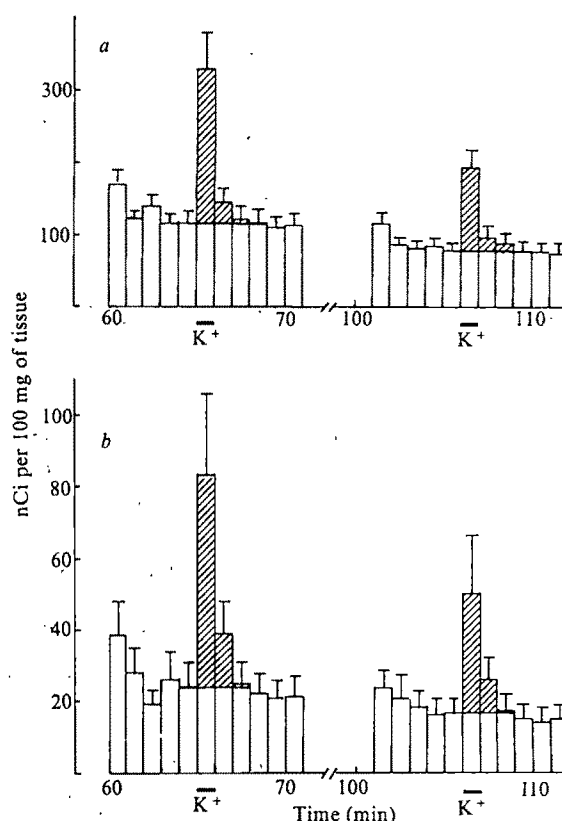


Fig. 1 Release of radioactivity from slices of rat corpus striatum and occipital cortex during exposure to 20 mM K⁺. Ordinate, fraction of the total radioactivity in the tissue released per min (nCi per 100 mg of tissue), abscissae, time (in min) after the end of the incubation with either ³H-noradrenaline (NA) or ³H-dopamine (DA). The open columns represent the spontaneous outflow of total radioactivity in 1-min samples. The horizontal bar indicates the 1-min exposure to 20 mM K⁺ which was repeated 40 min later. The shaded areas indicate the increase in release of radioactivity induced by K⁺. *a*, release of ³H-DA from slices of rat corpus striatum; values are the mean \pm s.e.m. ($n = 13$); *b*, release of ³H-NA from slices of rat occipital cortex, values shown are the mean \pm s.e.m. ($n = 7$). Tissue tritium content at the end of the experiment was $3,546 \pm 616$ nCi per 100 mg ($n = 13$) in the striatum and 938 ± 98 nCi per 100 mg ($n = 7$) in the occipital cortex; n = number of experiments per group.

potassium was expressed as per cent of the tissue stores: total nCi released as a fraction of the total radioactivity present in the tissue at the onset of stimulation⁹. Figure 1 shows the pattern of ³H-transmitter release elicited by each of the two consecutive periods of exposure to potassium in the rat striatum (Fig. 1*a*) and in rat occipital cortex slices (Fig. 1*b*).

Table 1 shows that in contrast with the cerebral cortex slices,

Table 1 Influence of the calcium concentration in the medium on the potassium-induced release of ³H-transmitter from slices of rat occipital cortex or corpus striatum

Tissue	<i>n</i>	Per cent tissue stores*		<i>S</i> ₁ as % of <i>S</i> ₂
		<i>S</i> ₁	<i>S</i> ₂	
		Ca ₂ ⁺ = 1.78 mM		
Occipital cortex	7	3.88 ± 0.74	4.63 ± 1.27	96.43 ± 9.19
Striatum	13	4.31 ± 0.68	5.14 ± 0.86	100.54 ± 18.18
Ca ²⁺ = 0 mM				
Occipital cortex	4	0.19 ± 0.06	4.06 ± 1.45	5.27 ± 1.08†
Striatum	8	1.97 ± 0.32	7.97 ± 2.09	38.01 ± 12.70†

*Per cent of the total tissue radioactivity released by exposure to 20 mM K⁺;

*S*₁ corresponds to the first 1-min exposure to K⁺ and

*S*₂ to the second, obtained 40 min after *S*₁.

Upper part, Tyrode solution with normal calcium; lower part, calcium-free medium during *S*₁.

Shown are mean values \pm s.e.m.; n = no. of experiments.

$^\dagger P < 0.001$ $^\ddagger P < 0.02$ when compared against the corresponding controls (upper part of the table).

the release of the labelled transmitter elicited by K^+ from the rat striatum was not entirely calcium-dependent. In the absence of calcium, the release of 3H -noradrenaline induced by potassium from the cerebral cortex was practically abolished (Table 1). In the striatum, however, as much as 40% of the release elicited by K^+ was found to be calcium-independent. Figure 2 shows that morphine and β -endorphin significantly decreased the K^+ -induced release of 3H -noradrenaline from the cerebral cortex. The effects of both opiate receptor agonists were reversed in the presence of naloxone (Fig. 2). In contrast with these results, neither morphine nor β -endorphin were able to inhibit the release of 3H -dopamine from rat striatal slices (Fig. 3) even when tested in concentrations higher than those found to be effective in the cerebral cortex (compare Figs 2 and 3).

Our results support the view that presynaptic inhibitory opiate receptors are present in noradrenergic nerve endings of the rat cerebral cortex. In addition, we found that β -endorphin stimulates these presynaptic opiate receptors and that it is at least 10 times more potent than morphine in reducing 3H -noradrenaline release. The failure of morphine and of β -endorphin to reduce the release of 3H -dopamine from the striatum represents a conflict of evidence with a previous report⁶. It should be noted, however, that in our experimental conditions, we stimulated release with a 1 min exposure to 20 mM K^+ , while in the previous publication, 53 mM K^+ during 20 min was used⁶. We consider our experimental conditions (short pulse of a moderate potassium concentration) to mimic nerve stimulation more closely than a prolonged exposure to a rather high concentration of K^+ .

During a 1 min exposure to 20 mM K^+ , approximately 5% of the total tissue radioactivity was released from the striatum (Table 1). In separate experiments in which the slices were exposed to 40 mM K^+ for 5 min, $36.27 \pm 2.84\%$ of the total tissue radioactivity was released from the striatum (mean \pm s.e.m. of four experiments). We consider that such a massive release of 3H -dopamine is not an adequate model for studies on transmitter release. It should be noted that for similar studies in the peripheral nervous system the fraction of the total tissue radioactivity released by nerve stimulation does not exceed 5% of the tissue stores⁹⁻¹². Attention should be drawn to the fact that even for a short lasting exposure to as little as 20 mM K^+ , more than one-third of the labelled transmitter released from the striatum represented a calcium-independent release. Under similar experimental conditions, the release of 3H -noradrenaline from the rat cortical slices was entirely calcium-dependent.

Fig. 2 Effects of morphine and β -endorphin on the release of 3H -noradrenaline elicited by potassium in the rat occipital cortex. Ordinate, ratio of fractional release (S_2/S_1); S_1 corresponds to the first 1-min exposure to 20 mM K^+ and S_2 to the second obtained 40 min after S_1 . Morphine was added 25 min before S_2 ; β -endorphin 10 min before S_2 and naloxone 35 min before S_2 . Values shown are mean \pm s.e.m. of 4-7 experiments per group.

* $P < 0.001$ when compared against the untreated group.

† $P < 0.001$ when compared against morphine 10 μM .

‡ $P < 0.005$ when compared against β -endorphin 0.6 μM .

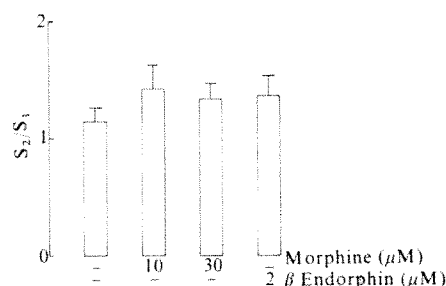
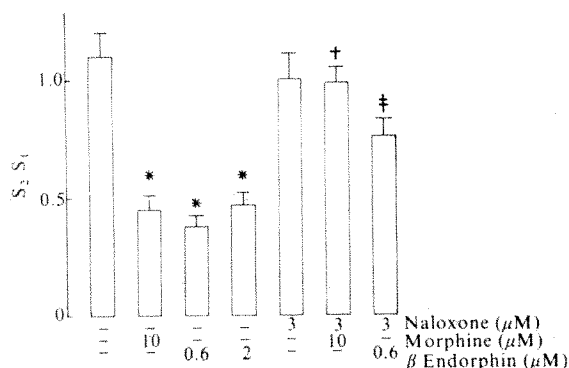


Fig. 3 Effects of morphine and β -endorphin on the release of 3H -dopamine elicited by potassium in rat striatal slices. Ordinate, ratio of fractional release (S_2/S_1); S_1 corresponds to the first 1-min exposure to 20 mM K^+ and S_2 to the second obtained 40 min after S_1 . Morphine was added to the medium 25 min before S_2 , and β -endorphin 10 min before S_2 . Values shown are mean \pm s.e.m. of 6-11 experiments per group.

Our results regarding the 40% calcium-independent release of 3H -dopamine from the striatum are in disagreement with previous reports which demonstrated either total calcium dependence^{13,14} or only a 15% calcium-independent component¹⁵, although a 33% calcium-independent component for the potassium-induced release of 3H -dopamine from the rat striatum was reported by Dismukes and Mulder¹⁶. We have found that the extent of the calcium-independent component for 3H -dopamine release from the striatum (40%) was not affected by inhibition of monoamine oxidase *in vitro* or *in vivo* with pargyline. Under our experimental conditions 3H -dopamine release originated from reserpine-sensitive storage sites in the striatum. In support of this view it was found that pretreatment with reserpine (5 mg kg⁻¹, 24 h before the experiment) reduced considerably the ability of the striatum to take up and store 3H -dopamine. In these experiments, exposure to K^+ was practically ineffective in eliciting 3H -dopamine release. It is concluded that β -endorphin and morphine reduce noradrenaline release from the rat cerebral cortex through the stimulation of presynaptic opiate receptors. Neither morphine nor β -endorphin reduced the release of dopamine from the rat striatum. In addition, a significant calcium-independent release of 3H -dopamine has been found in the rat striatum.

The magnitude of the calcium-independent component of 3H -dopamine release may vary with the strength and duration of the depolarising stimuli. Consequently, it is possible that the recent controversial reports on the effects of dopamine agonists and neuroleptics on dopamine release from the rat striatum may reflect experimental conditions with different degrees of calcium-independent release of 3H -dopamine^{13-14,16-18}. As a result of these conflicting reports, the presence of presynaptic dopaminergic receptors and their role in the regulation of dopamine release from nerve endings in the central nervous system remains an open question¹⁹.

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Received 19 September; accepted 19 December 1977.

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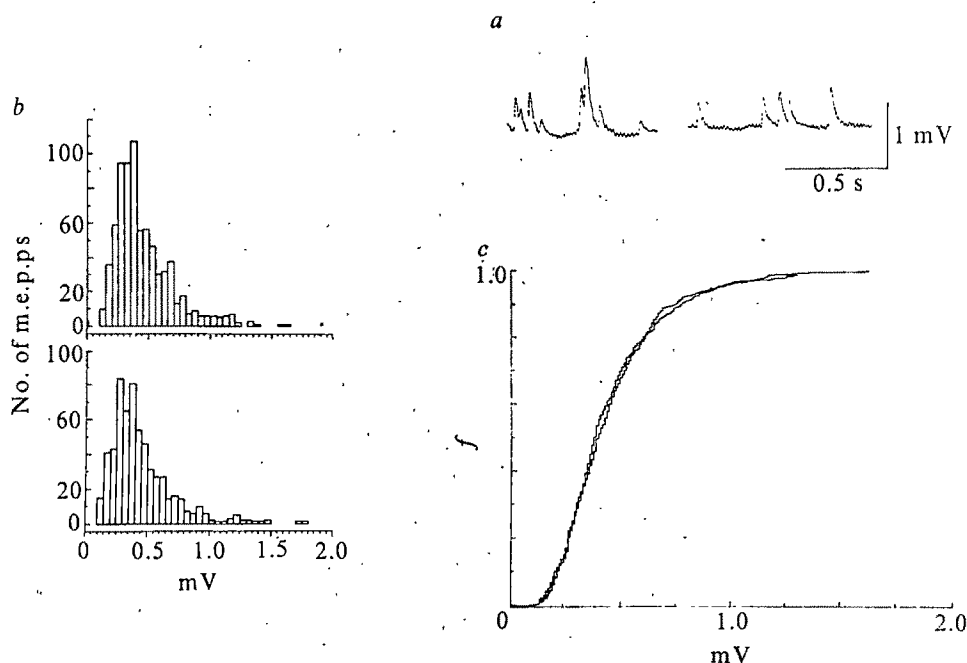
ACETYLCHOLINE (ACh) is released from stimulated nerve terminals in packets or quanta, each containing roughly 10,000 molecules^{1,2}, but we still do not know how the quanta are formed. A widely accepted hypothesis is that the ACh contained in vesicles found in the nerve terminal is released by exocytosis, while an alternative suggestion is the gated channel hypothesis. This takes into account the fact that the ACh which seems to be in free solution in millimolar concentrations in the terminals⁴ is probably the source of the ACh which leaks molecule by molecule from the resting terminals (only a small fraction of the spontaneous release is quantal^{5,6}). Other evidence against the vesicle hypothesis is that when nerves are exposed to radioactive precursors, the free ACh is labelled first, and with stimulation the newly synthesised ACh is released preferentially⁷. The free ACh could also be released in packets if stimulation opens gates on channels through which ACh diffuses from the terminals; with each opening of a channel, about 10,000 transmitter molecules might escape by moving out of the terminal down a concentration gradient. One way to test the gated channel hypothesis is to change abruptly the concentration of free ACh in the terminal, and so change the number of ACh molecules per quantum. This has been achieved in a molluscan cholinergic neurone by injecting acetylcholinesterase into the cell body. The enzyme diffuses down to the terminals, hydrolyses the free ACh, and produces a transmission block⁸. Unfortunately, in this preparation it is not possible to determine the quantal size, so it is not certain that the block occurs because the quanta become smaller. Quantal size is readily estimated at the vertebrate neuromuscular junction, but it is not possible to inject substances into the fine nerve terminals and there does not seem to be a feasible way to

To avoid problems with changing numbers of quanta released with each stimulus, spontaneously occurring miniature endplate potentials (m.e.p.s) were used to estimate quantal size. The protocol for the first set of experiments was as follows; a sciatic nerve-sartorius muscle preparation from the frog *Rana pipiens* was immersed in Ringer solution made hypertonic by the addition of 250 or 500 mM sucrose. After a one hour equilibration a microelectrode was inserted at the endplate and m.e.p.s were recorded. The microelectrode was then withdrawn until it was just outside of the fibre, the extracellular solution was aspirated from the bath, a fresh solution of normal tonicity was passed over the preparation, the micro-electrode was reinserted, and m.e.p.s were recorded once again. The change in solution and repenetration took less than 20 s. The m.e.p.p. amplitudes were measured before and after the change in tonicity.

Successful experiments met a set of criteria; (1) the fibre was seen clearly and visual landmarks were good, so the probability was high that the microelectrode was reinserted into the same fibre; (2) the membrane potential did not deviate by more than 3 mV during the penetrations or with the repenetration, so the amplitudes did not have to be corrected for changes in membrane potential; (3) m.e.p.p. frequency was sufficiently low for some individual m.e.p.ps to be distinctly separated in time from their fellows, so that their amplitudes could be accurately measured. This criterion might seem to be impossible to meet. Hypertonic solutions are known to cause massive increases in m.e.p.p. frequency, an increase in tonicity of 100 to 150 mOsm often produces frequencies too high for the accurate counting of events; the records are far too jumbled for accurate amplitude measurements. It has been found, however, that with increases in tonicity above 450 mOsm l^{-1} m.e.p.p. frequency begins to decline once again¹¹. Therefore, in Ringer containing 250 or 500 mM sucrose, some junctions can be found at which there are sufficient numbers of free-standing m.e.p.ps for accurate amplitude measurements.

From many attempts, seven experiments met the three criteria. An example is illustrated in Fig. 1. There is no significant difference between the mean amplitudes in hypertonic and in normal Ringer's solution. In both solutions, the amplitude

Fig. 1 *a*, Examples of m.e.p.ps recorded in Ringer (right hand trace) and in Ringer plus 500 mM sucrose (left hand trace). The Ringer contained (in mM): 110 NaCl, 2.0 KCl, 2.5 CaCl₂, 6 Na-Ntris- (hydroxymethyl) methyl-2-aminoethanesulphonic acid (TES) at pH 7.4 and 10⁻⁶ g ml⁻¹ neostigmine methylsulphate. Resting potential -78 mV. The m.e.p.ps were recorded on tape at 7½ s⁻¹ and played back at 1½ inch s⁻¹ to a Hewlett Packard ink writer; *b*, amplitude histograms of the m.e.p.ps recorded in one experiment in Ringer (mean ± s.e.m. 0.45 ± 0.24, *n* = 755 (upper trace) and in Ringer plus 500 mM sucrose (0.47 ± 0.26, *n* = 665, lower trace); *c*, the cumulative distribution of amplitudes in the two solutions. The cumulative relative frequency, *f*, is plotted as a function of the amplitudes. The probability of the deviation between the two distributions occurring by chance is greater than 0.15.



distribution is skewed toward higher values and neither fits a normal probability distribution function. Therefore, the two distributions were compared further by plotting the cumulative probability distribution functions of the amplitudes and then comparing the two by the Kolmogorov-Smirnov statistic. The differences between the two are readily attributed to chance variation ($P > 0.1$). The records were carefully examined by eye to see if there was a transient change in amplitude immediately following the solution change, but none was detected. Furthermore, the regression line for the first 50 or 100 m.e.p.p. amplitudes following the solution change were calculated. The slopes of the lines were indistinguishable from zero (for 100 events in the experiment in Fig. 1, $b = 0.0001 \pm 0.00027$). Similar results were obtained in the six other successful experiments (in two of these the transition was in the opposite direction, from normal to hypertonic Ringer).

Before concluding that changes in terminal volume have no effect on quantal size, it is necessary to rule out the possibility that changes in tonicity have other, compensating effects on endplate physiology, so that real changes in the amounts of ACh released are not detected in the experiments. An increase in tonicity might decrease the sensitivity of the endplate to ACh, then more ACh could be released in the hypertonic solution without producing an obvious increase in amplitude. Nastuk and Parsons¹² studied the depolarisation produced by 0.011 mM carbamylcholine in normal Ringer and in Ringer plus 300 mM sucrose, and concluded that the sensitivity was unchanged. Another possibility is that the change in muscle fibre volume produces substantial changes in the cable properties of the endplate region. If this occurred, transient, identical pulses of ACh might generate the same conductance change and current flow at the endplate, and nonetheless give m.e.p.s of different amplitudes because of altered cable properties. The initial experiments suggested that substantial changes in cable properties are unlikely, because the shape of the m.e.p.s is so similar in the normal and in the hypertonic solutions. This was checked by eye and also by measuring the half-times for the decay of the m.e.p.s (in the example shown in Fig. 1, normal Ringer = 13.3 ± 0.46 ms, $n = 22$; hypertonic Ringer = 13.2 ± 0.57 ms, $n = 29$).

This possibility was tested further by using the two micro-electrode method for voltage clamping the endplate, so that the amplitudes of the miniature endplate currents (m.e.p.s) could be measured¹³. The disadvantage of this method is that there is a somewhat greater delay between the solution change and the reinsertion of two electrodes than in the previous experiments; in other respects the protocols were identical.

Figure 2 shows m.e.p.s at various holding potentials at the same endplate in normal and in hypertonic solution. The m.e.p.s have a slightly lower mean amplitude in hypertonic than in normal Ringer. (This result might be anticipated because the muscle fibre will also shrink in the hypertonic solution, increasing $[K]_i$ and $[Na]_i$, thereby making the reversal potential for the e.p.p. somewhat more negative. But this explanation glosses over a complicated series of possibilities. For example, the change in intracellular ion concentrations will change the surface potential on the inner side of the sarcolemma¹⁴, and this would be expected to change membrane ionic conductances.) The important point is that there was no indication of even a transient increase in the m.e.p.s following the transition from hypertonic to normal Ringer, nor was there any notable change in the time course of the m.e.p.c. Similar results were obtained in two additional voltage clamp experiments.

Thus, abrupt change in tonicity, which must produce almost stepwise alterations in nerve terminal volume, do not change m.e.p.p. amplitudes or m.e.p.s in the direction expected if the amount of ACh released per quantum depends on the concentration of free ACh in the cytoplasm of the terminal. From the present experiments it is still conceivable that quantal size depends upon free ACh, but this requires that following a substantial change in concentration—probably at least two-fold

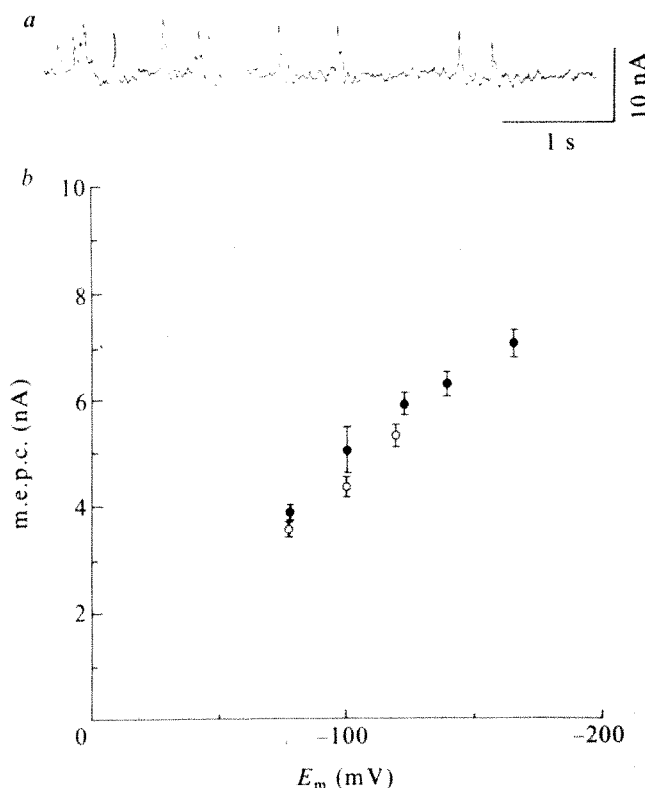


Fig. 2 *a*, Examples of the m.e.p.s in Ringer (right hand trace) and in Ringer plus sucrose (left hand trace) at a holding potential of -121 mV. The solutions and the method of data collection are given in the legend to Fig. 1. *b*, M.e.p.s recorded in a preparation in Ringer (●) and in Ringer plus 500 mM sucrose (○). The vertical bars indicate \pm the s.e.m.

—the terminal is capable of readjusting to normal levels of ACh within one or two minutes. When these observations are taken together with the evidence that quantal size does not vary with changes in the membrane potential of the nerve terminals¹⁵, as would be expected if free ACh were released as a cation through a channel, then the balance is overwhelmingly in favour of a release mechanism which does not depend upon the free ACh concentration. The vesicle hypothesis, with suitable modifications to account for such observations as the preferential release of newly synthesised transmitter, offers a most reasonable alternative.

This work was supported by grant NS-10320 from the National Institute of Neurological and Communicative Diseases and Stroke.

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Received 14 November; accepted 9 December 1977.

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Benzodiazepines specifically modulate GABA-mediated postsynaptic inhibition in cultured mammalian neurones

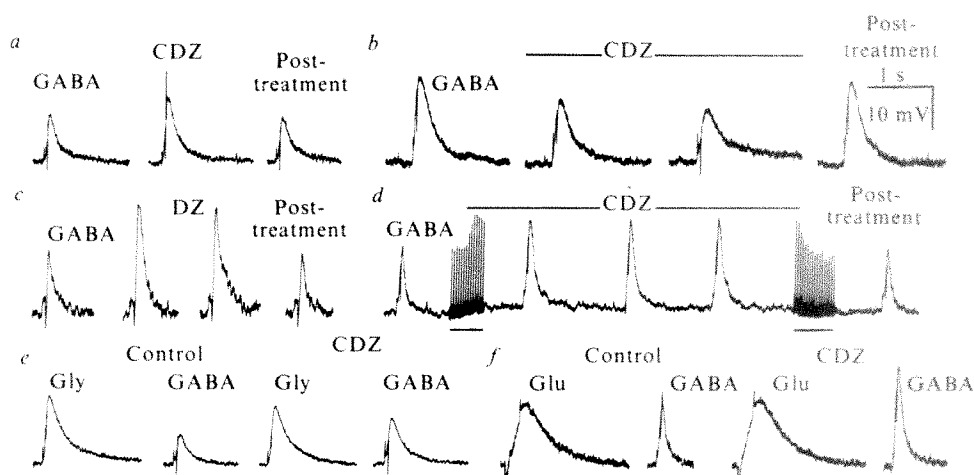
BENZODIAZEPINES (BDZ) such as diazepam (DZ) and chlordiazepoxide (CDZ) are commonly used anticonvulsants, muscle relaxants, antianxiety agents and hypnotics. The cellular mechanisms underlying these clinically important effects have not been established, but attention has been focused on the effect of BDZ on pre- and postsynaptic responses to the putative neurotransmitter amino acids, specifically glycine (Gly)^{1,2} and γ -aminobutyric acid (GABA)³⁻⁷. An effect of BDZ on postsynaptic Gly receptors was suggested by the finding that BDZ reduce strychnine binding to central nervous system (CNS) synaptic membrane fractions *in vitro*², but physiological studies, *in vivo*, have provided little support for such an interaction with Gly³. BDZ have been reported (1) to facilitate presynaptic inhibition in the spinal cord^{4,5}, at least partially GABA-mediated⁸⁻¹⁰; (2) to facilitate GABA-mediated pre- and postsynaptic inhibition in the cuneate nucleus⁶; (3) to mimic the presynaptic action of GABA on preganglionic nerve terminals¹², and (4) to antagonise^{13,14} and to enhance^{15,16} the postsynaptic action of GABA on CNS neurones. It has also been suggested that BDZ alter GABA metabolism^{4,5} or mobilise GABA from neuronal storage sites¹⁷, and BDZ have been demonstrated to bind specific receptors on synaptosomal membrane preparations derived from rat brain¹⁸, an effect which is independent of amino acid receptors. Thus, it is uncertain whether BDZ action is GABA or Gly receptor-mediated, due to an alteration of GABA metabolism, or due to direct binding to a specific receptor which binds BDZ. We have studied the effect of DZ and CDZ on amino acid-mediated postsynaptic responses in cultured mammalian spinal cord neurones and report that the BDZ selectively modify GABA-mediated postsynaptic inhibition in a dose-dependent fashion, augmenting the response at low doses and antagonising the response at higher doses.

Cultures were prepared from spinal cords removed from 13-14-d-old foetal mice as described previously¹⁹, and were grown on collagen-coated 35-mm dishes for 5-8 weeks before the study. Electrophysiological recordings were made on a

modified stage of an inverted phase microscope at 37 °C. All recordings were made in normal growth medium (90% minimal Eagle's medium/10% horse serum) to which $MgCl_2$ was added (final Mg^{2+} concentration of 10 mM) to suppress spontaneous activity and allow a clearer recording of postsynaptic responses. BDZ and amino acids were iontophored using a constant current device and high resistance 8-tube micropipettes (50-100 M Ω) filled with GABA (0.5 M, pH 3.5), Gly (0.5 M, pH 3.5), glutamate (Glu) (0.5 M, pH 8.6), (all from Sigma), DZ (10 mM, pH 3.5) and CDZ (100 mM, pH 3.5) (Hoffman-Roche, Nutley, New Jersey).

Both CDZ (Fig. 1a, d) (15 cells) and DZ (Fig. 1c) (6 cells) rapidly and reversibly augmented the response elicited by the iontophoresis of GABA (Fig. 1d). This augmentation could frequently be produced by simple diffusion of BDZ from the pipette tip but usually required passage of 1-5 nA of positive current. When augmentation was achieved, if additional current (1-10 nA) was passed through the BDZ pipette, antagonism of the GABA response was produced (Fig. 1b). The entire range of augmentation and inhibition was usually obtained without any alteration in resting membrane potential or input resistance, but a direct effect of BDZ was occasionally obtained (five cells), consisting of depolarisation and increase in conductance (not illustrated). The effect of these agents was specific for GABA since the responses to Gly (Fig. 1e) and Glu (Fig. 1f) were not augmented by CDZ application despite clear augmentation of GABA responses (five cells). The nature of this action of BDZ on the GABA response is unclear. One possible mechanism for augmentation of the response could involve an inhibition of the uptake of GABA, thus prolonging GABA synaptic action. This is unlikely as such an effect would be expected to prolong the time course of the response and this does not occur either with augmentation (Fig. 2a) or with antagonism (Fig. 2b), and would not explain the high dose antagonism of the response. The specificity of the interaction between BDZ and GABA suggests, however, that a direct effect of BDZ on the Cl^- conductance mechanism used by GABA and Gly²⁰ is unlikely; rather, the most likely site of action is at the GABA receptor with a modification of the affinity of the receptor for GABA. Such a conclusion is supported by initial analysis of the augmenting effect of CDZ on GABA dose-response curves; this effect is associated with a parallel shift to the left without alteration of the V_{max} para-

Fig. 1 Effect of benzodiazepines on amino acid responses in cultured mammalian spinal cord neurones. Intracellular recordings were made using 3M KCl micropipettes (25-40 M Ω) with the membrane potential hyperpolarised to -90 mV. Amino acid responses were elicited with 50 ms pulses of appropriate polarity while BDZ were applied with a steady cationic current. Under these conditions, the reversal potential for all amino acid responses is more positive than -30 mV, and thus all responses are depolarising. CDZ [as] and DZ [cs] reversibly augment the GABA response at low iontophoretic currents and reversibly depress the GABA response at higher currents [bs] (illustrated for CDZ only). A continuous record of augmentation of GABA responses by CDZ is shown in d. The penwriter is slowed (see bars below d) at onset and offset of CDZ current (time calibration bar, 5 s). The response to Gly (e) and Glu (f) are not augmented by CDZ despite clear augmentation of the GABA response. Calibration bars apply to all penwrite traces except as described in d.



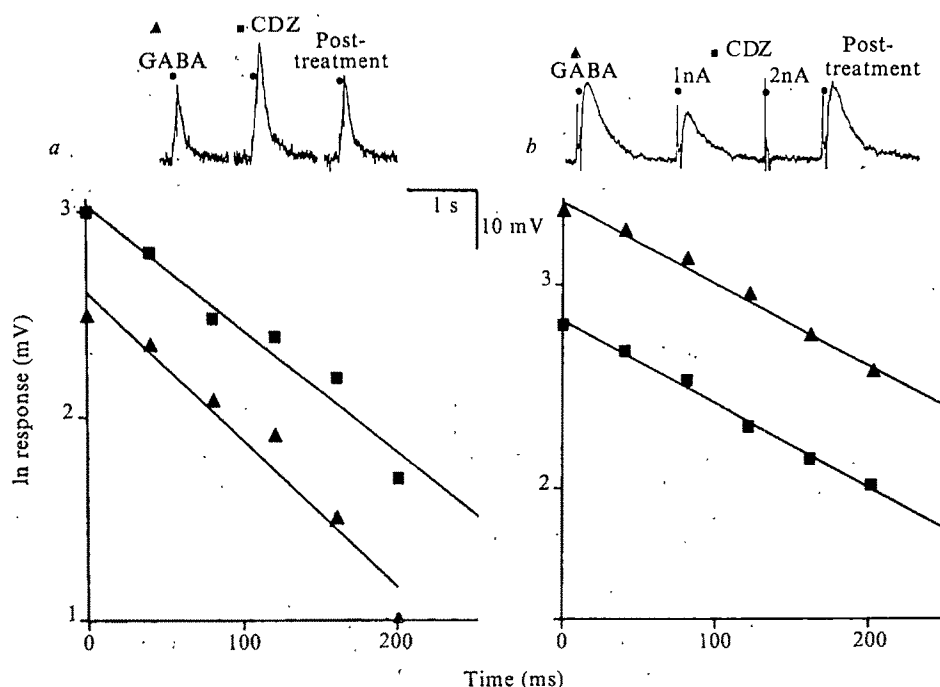


Fig. 2 Time course of GABA responses modulated by benzodiazepines. The effect of CDZ on the GABA response at low dose (a) and at higher dose (b) did not affect the time course of the GABA response as shown in semi-logarithmic plots of the amplitude of the GABA response following the peak response as a function of time.

meter in the Lineweaver-Burke plot (unpublished observations). Modification of coupling between receptor and ionophore is also a possible explanation; an effect on channel duration would be unlikely since this would modify the overall time course of the response, but an effect on unitary channel conductance could be a possible mechanism. Insufficient data is available to allow interpretation of BDZ antagonism of GABA responses.

Thus, this study directly demonstrates that the BDZ have a specific yet dose-dependent effect on GABA-mediated postsynaptic inhibition in mammalian spinal cord neurones, with augmentation obtained at low doses and depression elicited with higher doses. The results do not support BDZ enhancement of glycine-mediated postsynaptic inhibition, as has been suggested previously^{1,2}. Furthermore, the dual effect of BDZ on GABA responses may help to explain why previous studies on different preparations have found opposing results¹³⁻¹⁶. Augmentation of GABA-mediated pre- and postsynaptic inhibition may be the basic feature of its anticonvulsant activity since augmentation of GABA-mediated postsynaptic inhibition by the anticonvulsant phenobarbital has been observed in tissue cultured mammalian neurones^{20,22}, while diphenylhydantoin has been reported to prolong GABA responses in invertebrates²³. The therapeutic role of the inhibition of GABA responses by BDZ is not clear, but it may be responsible for some of the toxic side effects of CDZ and DZ such as ataxia, seizures and tremor. The role of the direct effect of BDZ on neuronal membrane properties seen in these experiments and in sympathetic preganglionic nerves^{11,12} and that of direct BDZ binding to specific receptors derived from rat brain¹⁸ is also uncertain. In the present study, the direct effects were observed at BDZ currents higher than those required for GABA response augmentation. Thus, if GABA response enhancement underlies its anticonvulsant action, the direct effects of BDZ may be relevant only at toxic or non-therapeutic doses.

A study published after submission of our manuscript showed that CDZ selectively augments GABA responses elicited from neurones in chick spinal cord cultures²⁴.

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Isolation of specific neurones by affinity methods

THE complex heterogeneity of cell populations in the nervous system severely limits the study of many important questions in neurobiology. Improvement of the current techniques of neuronal cell culture will require methods for selective isolation of neurones belonging to specific functional classes. Present methods include a degree of separation of neurones from non-neuronal cells by physical methods¹⁻³ but these differentiate only on the basis of size or density. The separation of classes of tumour cells and lymphocytes has been accomplished by affinity chromatography using lectins or antibodies against surface antigens⁴⁻⁷. In principle neurone purification could be approached in the same way. Among other surface identities,

Table 2 Induced and constitutive plasmid transfer

Donor strain	Mating medium	
	Arginine + pyruvate	Octopine
B6S3	$\leq 10^{-7}$	19×10^{-2}
B6S3OCC ^c 6	5×10^{-3}	8×10^{-2}
B6S3OCC ^c 8	$\leq 10^{-7}$	13×10^{-2}

Values are transconjugant frequencies amongst the recipients. Recipient and donor strains were grown in arginine plus pyruvate minimal medium (see Table 1) and the crosses were incubated on arginine plus pyruvate minimal medium or octopine minimal medium. The bacterial mixtures were deposited as drops directly on to the mating medium. The crosses were collected, after incubation for 48 h at 25 °C, with a platinum loop and the bacteria were plated on selective minimal medium (rifampicin 100 µg ml⁻¹, octopine 10 mM) after suspension and dilution in water. After incubation for 4 d at 25 °C the Petri dishes were examined and the colonies scored. The higher efficiency of transfer obtained in these crosses as compared with those in Table 1, indicate that these plate matings result in a more efficient conjugation.

opine found in octopine-containing tumours¹⁶, did not induce conjugative activity.

Experiments performed with some strains carrying nopaline Ti plasmids gave analogous results. Some strains such as K14 and T37 can transfer their plasmid in the presence of 10 mM nopaline. For instance, in a cross between strain K14 as the donor and strain GV3102 (a derivative of strain C58C1 that is resistant to 100 µg ml⁻¹ rifampicin and 100 µg ml⁻¹ erythromycin) performed in conditions analogous to those described in Table 1, some transfer of the Ti plasmid (about 10⁻⁴) was detected in the absence of nopaline, whereas 10⁻² of the recipients had acquired a Ti plasmid when the mating was performed in the presence of nopaline. On the other hand, the low frequency of Ti transfer by another nopaline strain, C58, was not enhanced by adding nopaline to the mating mixture.

As the catabolism of nopaline and octopine is coded for by plasmid genes^{7,17} and is also inducible by the same substrates, it was possible that both the catabolic activity and the transfer activity are under the control of a single regulatory system. One would then expect that both processes would respond to the same inducers, and that some regulatory mutations would be found that would interfere with both of these processes. Five natural opines are now known: octopine, nopaline, octopinic acid, lysopine and histopine. We found that the first four of them can induce both processes (ref. 18) and that the fifth is not an inducer for either process (A.P. and J.T., in preparation). To further explore the situation we performed crosses between strain GV3201 as a recipient and strains B6S3, B6S3OCC^c6 and B6S3OCC^c8, the latter two being spontaneous mutants of B6S3, constitutive for octopine catabolism¹⁸. (Strain GV3201 was derived as a rifampicin resistant mutant from strain B9105 (ref. 19) which was shown to be cured of the two plasmids harboured by the parental B91 strain.) The results shown in Table 2 demonstrate that octopine is required for transfer by B6S3 and B6S3OCC^c8, whereas for B6S3OCC^c6 it took place also in the absence of this inducer.

With the nopaline strain T37, whose Ti plasmid transfer is inducible by nopaline, a mutant, T37NOC^c1, could be isolated that is able to grow on octopine as sole carbon and nitrogen source and was shown to be constitutive for both Ti plasmid transfer and nopaline catabolism¹⁸. The capacity of these constitutive mutants to catabolise octopine seems to be due to the ability of the nopaline-degrading enzyme to use octopine as a substrate as well¹⁸. However, other independently isolated derivatives of the same strain, able to utilise both octopine and nopaline as growth substrate, did not transfer their Ti plasmid in the absence of inducer.

Mutants constitutive for transfer were also selected for

directly, by looking for conjugation without induction: high titre mixtures of donor strain GV3110 (C58C1(TiB6S3)) and recipient strain GV3103 (C58C1 Str^rSpc^r) were plated on 0.2% glucose minimal medium. After incubation for 48 h at 28 °C the mixture was inoculated first in a rich medium (0.5% Bacto-beef extract, 0.1% Bacto-yeast extract, 0.5% peptone, 0.5% sucrose in 0.002 M MgSO₄) with 100 µg ml⁻¹ spectinomycin and 100 µg ml⁻¹ streptomycin to eliminate the donor cells and subsequently in octopine minimal medium to enrich for transconjugants. The total transconjugants-recipient population thus obtained was used as donor in a second conjugation without induction with strain GV3102 (C58C1rif^rery^r) as recipient. In this second cross a high level of octopine-utilising transconjugants was obtained. These transconjugants, when used in further crosses, transferred their Ti plasmid at a high frequency (about 10⁻¹) in the absence of octopine and were thus transfer-constitutive.

These results are consistent with the hypothesis of a common regulatory gene controlling two distinct operons, one involved in catabolic activity and the other in plasmid transfer. Our results demonstrate clearly that conjugative activity of the Ti plasmids can be induced by the substrates of some specific enzymes coded for by the Ti plasmids. The types of regulatory mutants that we have been able to obtain thus far are consistent with the idea that a common regulator gene is involved in the control of the activity of two distinct functions—catabolism and transfer.

It is possible that this is but one example of a more general phenomenon. It could be that several bacterial plasmids can be induced to conjugate by a key substance that is a substrate of enzymes coded for by plasmid-borne genes. Some catabolic plasmids and possibly RTF factors might show similar behaviour. Induction of conjugative activity by these conditions that give a selective advantage to plasmid-harboured bacteria would result in efficient spread of the plasmid and thus in a more rapid adaptation of a bacterial population to changes in environmental conditions. Nakazawa and Yokota²⁰ have described a mutant TOL plasmid from *Pseudomonas putida* with increased constitutive levels of metapyrocatechase and increased conjugative activity. The behaviour of this mutant might be explained by the same type of simultaneous regulation of catabolic and transfer functions.

This work was supported by grants from the Australian Research Grants Committee and the Kankerfonds van de ASLK and grants from the Fonds voor Kollektief Fundamenteel Onderzoek to J.S. and M.V.M. M.H. is an Aangesteld Navorsers of the National Fonds voor Wetenschappelijk Onderzoek.

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Functional stabilisation of HeLa cell histone messenger RNAs injected into *Xenopus* oocytes by 3'-OH polyadenylation

WE have shown that, when injected into *Xenopus* oocytes, poly (A) free globin messenger RNA is translated for a short period of time and then rapidly degraded¹⁻³, while, under the same conditions, native mRNA shows a marked stability^{1,2,4}. The poly (A) segment itself is responsible for the stability of native globin mRNA, since its readdition to previously de-adenylated mRNA restores the stability of the message⁵. We have shown also that the poly (A) stretch must contain a minimum number of adenylate residues to ensure its protective function⁶. It was interesting to see whether the concept of the stabilisation of eukaryotic mRNAs by 3'-OH polyadenylation is general. This can be done by looking at the effect of the presence of a 3'-OH poly (A) segment on the stability of various mRNAs. Here we study the stability of HeLa cell poly (A)-free histone mRNAs injected into *Xenopus* oocytes, and the effect of 3'-OH polyadenylation on this stability.

An RNA fraction enriched in histone mRNAs (His mRNA) was obtained from polysomes of synchronised HeLa cells in S phase^{7,8}. This RNA preparation, which is not retained on poly (U)-Sepharose on 0.5M KCl at room temperature, directs exclusively the synthesis of human histones in a reticulocyte cell-free protein synthesising system^{7,8}. Fifty oocytes from a single

Xenopus laevis female were injected with 50 µl each of a 750 µg ml⁻¹ aqueous solution of His mRNA. At different times after injection, sets of ten oocytes were incubated in a saline medium containing 1 mCi ml⁻¹ of ³H-lysine (67 Ci mmol⁻¹) at 19 °C for several hours. At the end of the incubation periods, protein samples were prepared from oocytes and analysed by sodium dodecyl sulphate (SDS)-polyacrylamide slab gel electrophoresis¹⁰. Reference ¹⁴C-HeLa cell histones were used as a control. At the end of the electrophoresis, the gels were dried and submitted to fluorography¹¹.

Synthesis of histones H₃, H_{2b}, H_{2a} and H₄ is clearly detected in oocytes injected with the His mRNA fraction (Fig. 1). The synthesis rate of these four proteins is maximal from 5-10 h after microinjection; it then decreases rapidly and becomes almost undetectable after 20 h (Fig. 1). From this result, we conclude that four naturally poly (A)-lacking mRNAs (or mRNAs 'possessing a very short poly (A) segment, since they are not retained on poly (U)-Sepharose) behave, as far as their translation is concerned, in a very similar way to globin mRNA from which the poly (A) segment had been artificially removed¹. It thus seems that a 3'-OH poly (A) segment is required to ensure the stability of several eukaryotic mRNAs.

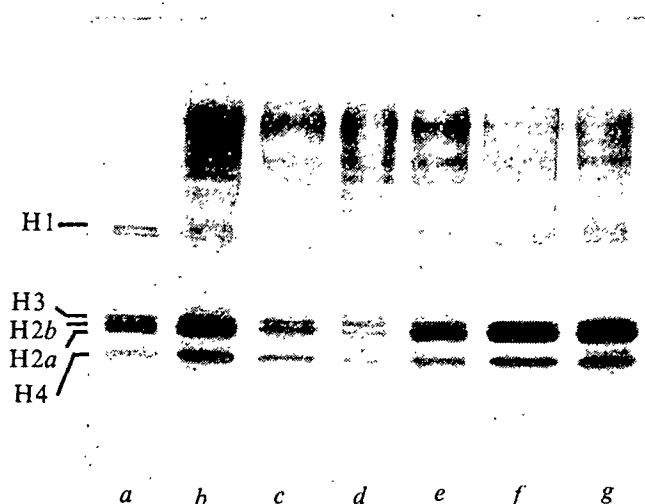
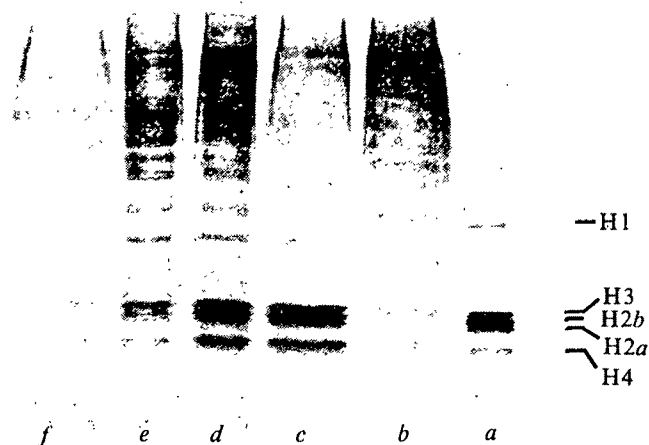


Fig. 1 Fluorography of a sodium dodecyl sulphate (SDS)-polyacrylamide gel electrophoresis of proteins from oocytes injected with a histone mRNA-enriched fraction from HeLa cells (750 µg ml⁻¹) (His mRNA). Fifty oocytes from a single *Xenopus laevis* female were injected with the His mRNA preparation. At different times after microinjection, sets of ten oocytes were incubated at 19 °C in Barth medium containing 1 mCi ml⁻¹ of ³H-lysine (67 Ci mmol⁻¹). At the end of the incubations, oocytes were washed and homogenised in Tris-glycine 0.05M, pH 8.9. Cell debris and yolk platelets were removed by low speed centrifugation and the supernatant was made 2% in SDS and 5% in mercaptoethanol. Aliquots of the supernatants containing 400,000 dpm of trichloroacetic acid precipitable counts were analysed by electrophoresis on a 15% polyacrylamide slab gel¹⁰. After staining and destaining, gels were treated for and submitted to fluorography¹¹. a, Reference ¹⁴C HeLa cells histones; b, proteins from control oocytes injected with water; c-f, proteins from oocytes injected with His mRNA. Incubation periods: b, c, 0-5 h; d, 5-10 h; e, 10-20 h; f, 20-48 h.

Fig. 2 Fluorography of a SDS-polyacrylamide gel electrophoresis of proteins from oocytes injected with either a native His mRNA fraction from HeLa cells (channels b-d) or this His mRNA fraction previously polyadenylated (channels e-g). Both RNA preparations have been injected at 750 µg ml⁻¹. Eighty oocytes from a single *Xenopus laevis* female were injected either with native His mRNA or with polyadenylated His mRNA (40 oocytes with each preparation). At different times after microinjection, sets of ten oocytes from each batch of cells were incubated at 19 °C in Barth medium containing 1 mCi ml⁻¹ of ³H-lysine (67 Ci mmol⁻¹). At the end of the incubations, oocytes were processed as described in Fig. 1 for gel electrophoresis. a, reference ¹⁴C HeLa cells histones; oocytes injected with native His mRNA; e-g, proteins from oocytes injected with polyadenylated His mRNA. Incubation periods: b, e, 0-7 h; c, f, 7-19 h; d, g, 43-60 h.



To test this assumption, a poly (A) stretch containing 40 to 50 adenylate residues was added to the 3'-OH end of the His mRNA fraction using an ATP : RNA adenylyltransferase from *Escherichia coli*, and the functional stability of this polyadenylated mRNA was tested after microinjection into oocytes. The polyadenylated His mRNA fraction and control poly (A)-free His mRNA were thus injected at a concentration of 750 µg ml⁻¹ into two sets of 50 oocytes from the same *Xenopus* female. At different times after microinjection, batches containing 10 oocytes were incubated in a saline medium containing 1 mCi ml⁻¹ of ³H-lysine (67 Ci mmol⁻¹) at 19 °C for several hours. At the end of the incubation periods, protein samples were prepared and analysed by SDS-polyacrylamide slab gel electrophoresis. At the end of the electrophoresis, the gels were dried and submitted to fluorography¹¹.



Fig. 1 *a*, Chick embryo sympathetic ganglion neurons bound to α BT-Sephadex 6MB beads. $\times 120$. *b*, The same beads do not bind HeLa cells significantly. α BT-derivatised beads were synthesised as follows; 100 μ l of *p*-aminobenzoylhexylamino Sephadex 6MB¹² beads were diazotised in 0.1 M sodium nitrite in 0.1 M HCl-0.2 M NaCl for 10 min at 0°C. The beads were washed with ice-cold PBS (after excess nitrous acid had been destroyed with 0.1 M sulphamic acid) and added immediately to a solution of approximately 1 mg of α BT in 0.5 ml of 0.05 M borax pH 8. Reaction was allowed to proceed overnight at 4°C and the resulting dark-yellow beads were washed extensively with PBS. The amount of toxin covalently attached to the beads was estimated by measuring the A_{280} of the supernatant at the end of the reaction. Typically a concentration of α BT on the beads of approximately 1 mM was achieved.

neurons have neurotransmitter receptors which provide one basis for functional classification and potentially provide targets for affinity probes of high specificity. As a model system we chose the chick embryo sympathetic ganglion neurons which are known to possess high numbers of the nicotinic type of acetylcholine (ACh) receptor³. These cells also possess high numbers of receptors for α -bungarotoxin

(α BT)⁹. We report here the successful separation by affinity chromatography of the principal neurones from sympathetic ganglia using α BT as a probe.

α BT was attached to Sephadex 6MB by reacting the toxin in borax buffer pH 8 with diazotised *p*-aminobenzoylhexylamino Sephadex 6MB (ref. 10). This procedure results in the formation of an azo linkage with one or other of the two histidine residues (positions 4 and 67)¹¹. In view of the structural similarity between types I and II neurotoxins¹¹ and the fact that the extra disulphide bond in type II toxins is not essential for activity¹² it is reasonable to assume a conformation for α BT similar to that recently determined for erabutoxin b by X-ray crystallography¹³. In this conformation the two histidines in α BT are well removed from the active site and their modification is unlikely to affect the biological activity. Indeed the derivatised beads bind chick embryo sympathetic ganglion neurones in preference to other cell types (Fig. 1).

Sympathetic ganglion cells were prepared from 19-d chick embryos as previously described¹⁴. At this age the neurones have approximately 2×10^5 α BT receptors per cell⁹. After trypsinisation (0.025%) the cells were dissociated in Puck's saline G containing 2 mg ml⁻¹ bovine serum albumin, 20 μ g ml⁻¹ deoxyribonuclease (DNase) and 10 units ml⁻¹ nerve growth factor (NGF)¹⁵; this will be referred to as medium A. The cells were then filtered through nylon gauze (pore size < 50 μ m), centrifuged and resuspended at 1×10^6 cells ml⁻¹ in medium A. The α BT-derivatised macrobeads were held in the bottom of a small glass water-jacketed column by a piece of nylon gauze held in place by a tightly fitting Teflon ring. The assembled column without beads was autoclaved and all manipulations were carried out in a sterile laminar flow cabinet. The beads were extensively washed with sterile phosphate-buffered saline (Ca²⁺ Mg²⁺-free Dulbecco's PBS), then medium A. The flow rate was adjusted to 1–2 ml h⁻¹ and the cell suspension loaded. It was important to maintain a temperature of 0–4°C throughout the isolation procedure for optimal viability of the neurones. The beads were washed until very few non-neuronal cells were present in the eluate. After further washing with PBS containing DNase (4 μ g ml⁻¹) and subsequently with PBS containing DNase and 0.1% trypsin, the column was clamped off, the temperature raised to 37°C and incubation allowed for 2–3 min. Foetal calf serum (FCS) was then added and the cells eluted with about 10 ml of F15 medium (GIBCO) containing 5% FCS (growth medium) into a sterile centrifuge tube. The cells were centrifuged, resuspended in 1 ml growth medium containing 10 U ml⁻¹ NGF and cultured.

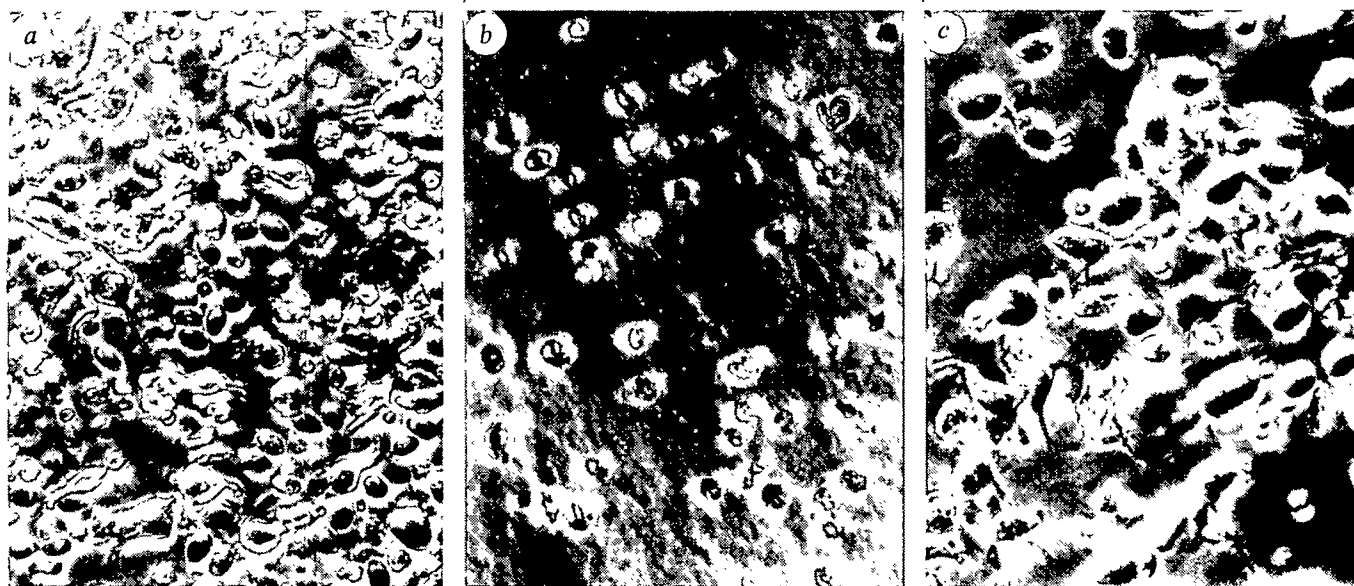


Fig. 2 Purification of sympathetic ganglion neurones. *a*, Original cell suspension containing approximately 10% principal ganglion neurones. *b*, Eluate from the affinity column during washing. Essentially all non-neuronal elements. *c*, Final suspension of purified neurones after removal from column by trypsin (0.1%). Phase contrast $\times 370$.

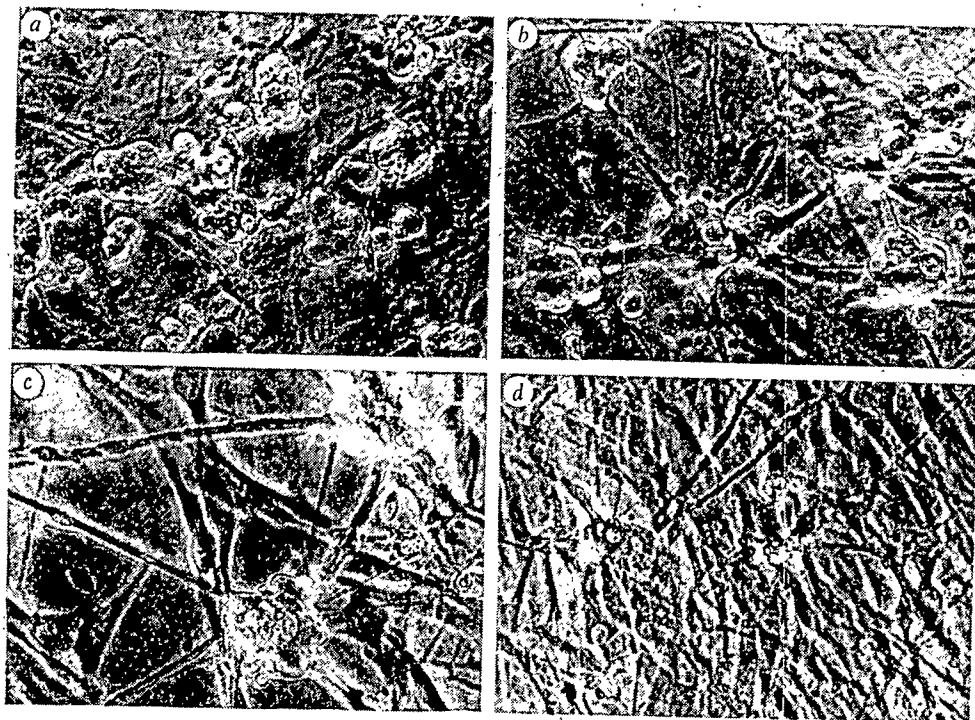


Fig. 3 Phase contrast photomicrographs of cultured sympathetic ganglion neurones: parallel purified and nonpurified cultures. $\times 328$. *a*, Purified neurones, 1 d *in vitro*. *b*, Purified neurones, 6 d *in vitro*. *c*, Non-purified neurones, 1 d *in vitro*. *d*, Non-purified neurones, 6 d *in vitro*. Comparison of (*b*) and (*d*) shows that the purified neurones are relatively free of fibroblasts while non-purified cultures are virtually overgrown by non-neuronal elements. The cells were plated into 33-mm tissue culture dishes (Kayline Plastics). For isolated neurones, plates were coated with tail collagen¹⁷. A collagen substrate is not necessary in the presence of non-neuronal cells. Cultures were maintained in a humidified atmosphere of 5% CO₂-95% air and the medium was changed every second day.

Figure 2 shows the cell suspension at different stages during the purification. The original cell suspension contained about 10% principal neurones and the final cell suspension > 95% neurones. Thus we have achieved a purification of the neurones of 9 to 10 fold. By the concomitant use of differential plating¹⁶ and the use of mitotic inhibitors for a short period after plating, the few remaining non-neuronal cells could be eliminated, giving 100% purity. Alternatively, nonspecific binding may be eliminated by more extensive washing or possibly by the inclusion of a small inert charged molecule to eliminate charge interactions.

The isolated neurones were viable after 6–8 h on the column. Cultured, purified neurones are compared with parallel non-purified cultures in Fig. 3. After the same period *in vitro* those neurones which had gone through the affinity column were still free of non-neuronal cells.

The functional viability of the isolated neurones was examined by electrophysiological measurements (Fig. 4). The neurones responded in an all-or-none fashion to electrical stimulation and responded to iontophoretically-applied acetylcholine, showing desensitisation on repeated application. Autoradiography showed that essentially all the isolated neurones bind [¹²⁵I]- α -bungarotoxin (data not shown).

Thus, we have been able to obtain essentially pure, viable and electrically active neurones. The same results were obtained using as an affinity probe the α -neurotoxin from the venom of *Naja nigricollis* purified by CM cellulose chromatography. The availability of suitable specific immobilised ligands for in-

vestigation *in vitro* of important questions such as synaptic specificity, neuronal-non-neuronal cell interactions and the behaviour of particular neurone populations in certain disease states. We are investigating the possibility of using small molecule affinity probes and applying our isolation procedure to neurones from the brain.

We thank John Leah for carrying out the electrophysiological measurements. This work was supported by the Australian Research Grants Committee.

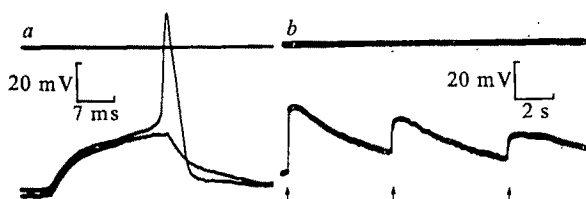
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Fig. 4 Intracellular recordings from purified chick embryo sympathetic ganglion neurones, 6 d *in vitro*. *a*, All-or-none response to a depolarising current pulse of 0.2 nA. *b*, Response to successive iontophoretic applications of acetylcholine (arrows). Ejecting current 75 nA, 70 ms. Electrical activity was monitored as previously described¹⁴.



Paired helical filaments of the Alzheimer type in cultured neurones

ALZHEIMER'S disease, a common progressive dementia of later life, is associated with several histopathological changes. Although one of these pathological changes, neuritic plaques with amyloid cores, has been reproduced in brain of mice following inoculation with scrapie agent^{1,2}, Alzheimer-type neurofibrillary degeneration has not yet been reproduced in the laboratory. This unique form of human neurofibrillary degeneration is composed of arrays of paired 100-Å diameter filaments twisted into a helical configuration with a period of approximately 800 Å (refs 3, 4). We present here evidence that paired helical filaments

morphologically closely resembling those found in the human disease are induced in cultured human foetal cerebral cortical neurones after exposure to an extract prepared from Alzheimer-affected brain.

Explanted fragments (0.5 mm^3) of cerebral cortex derived from two human foetuses of approximately 12–14 weeks gestation were used in this initial study. Explant cultures were maintained on collagen-coated coverslips⁵ in a medium composed of 35% Simm's balanced salt solution, 40% heat-inactivated human cord serum and 25% medium 199 (GIBCO), with 600 mg% glucose (final concentration). The coverslips with cultures were placed into plastic Petri dishes (Falcon) and incubated at 36°C in a water-saturated atmosphere of 95% air and 5% CO_2 . The culture medium was changed twice weekly. A saline extract was prepared from cerebral cortex of a patient who died aged 63 after a 6-yr history of progressive intellectual deterioration and whose brain exhibited widespread neurofibrillary degeneration and senile plaques typical of Alzheimer's disease. The brain was removed 12 h post-mortem and stored frozen at -90°C . Four months later a cortical sample of approximately 2 cm^3 was removed from the tip of the temporal lobe, thawed and homogenised in an equal volume of sterile physiological saline at 4°C in a Potter-Elvehjem homogeniser. The homogenate was spun in a bench-top centrifuge at 1,500 r.p.m., the pellet discarded, and the cell-free supernatant passed through a Millipore filter of 4,500 Å pore size. Ultrastructural examination of the filtrate by standard positive and negative staining methods did not reveal fragments of paired helical filaments. The filtrate did, however, contain fragments of microtubules, ranging in length from 600 to 2,300 Å and small membrane fragments. No particles

Fig. 1 Electron micrograph of an unaffected human foetal neurone representative of the culture system used in the study; 35 d *in vitro*. Arrowhead identifies presynaptic terminal of an axosomatic contact. Scale bar, $0.5 \mu\text{m}$.

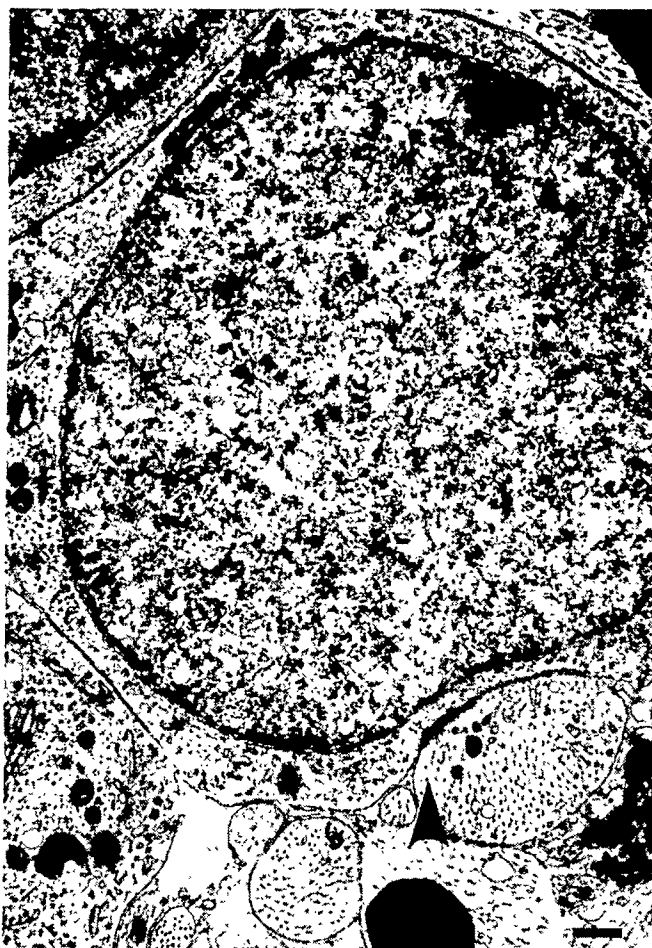


Fig. 2 Electron micrographs of paired helical filaments observed in foetal brain cultures exposed to an extract of Alzheimer affected brain; 35 d *in vitro*. a, Low power aspect of process with paired helical filament (arrowhead), enlarged in b; c, d, additional examples. Scale bars, $0.1 \mu\text{m}$.

exhibiting viral morphology were encountered. The human, foetal cerebral explants were exposed to an approximately 5% solution of the Alzheimer brain extract for the first 6 d in culture. After this initial exposure, feeding was carried out with pure culture medium. The cultures were fixed at various intervals after inoculation with 3.5% glutaraldehyde in phosphate buffer.

Detailed ultrastructural examination of cultures fixed after 4, 8 and 11 d of incubation revealed no paired helical filaments and the morphological characteristics of the cells were identical to those in control cultures (Fig. 1). However, after 14 d incubation, occasional paired helical filaments were observed, and after 35 d of incubation, paired helical filaments were found in many cell processes. Approximately 3% of all cell processes ($n = 491$) at 35 d incubation contained one or more paired helical filaments. Synaptic contacts established that many of the processes containing these paired filaments were definitely of neuronal origin. Many examples of paired helical filaments occurred in cells in which the cytoplasm seemed swollen and the density of organelles was reduced. The plasma membranes of affected cells were intact, although microtubules seemed fragmented and mitochondria were often dense. Many of the paired helical filaments seemed to be assembled from separate, morphologically normal appearing neurofilaments (Fig. 2d). The average diameter of the individual filaments composing the helical structures in the cultured cells was 95 Å (s.d. 16 Å , $n = 12$) and the average width of pairs was 289 Å (s.d. 45 Å , $n = 13$). This is to be compared with the

paired helical filament in Alzheimer's disease in which individual filaments have an average diameter of 95 Å (s.d. 17 Å, $n = 13$) and a mean overall width of 241 Å (s.d. 55, $n = 9$). The paired, helical filament of Alzheimer's disease usually exhibits nodes with a mean period of approximately 800 Å, although internode distances of up to 1,000 Å are not uncommon⁶. In the cultured foetal cells, the internode period ranged from 800 to 1,600 Å, and a mean value of 846 Å (s.d. 133 Å) was measured on several typical paired helical filaments displaying up to 10 nodes (Fig. 3c). In contrast to the neurofibrillary tangle of Alzheimer's disease, in which dense aggregates of paired helical filaments occur, the number of paired helical filaments per cultured cell was small, and dense aggregates were not observed. Typically, the paired helical structures induced in culture did not display electron dense material along the filaments and seemed more distinct than those observed in Alzheimer's disease.

Electron microscopic examination of over 500 human cerebral cultures not exposed to the Alzheimer extract has never revealed paired helical filaments, although these cultures were exposed to a variety of toxic agents, including aluminium⁷, osmotic stress, fungi and bacteria. Moreover, over 500 brain explants from goldfish⁸, foetal rabbits and foetal mice similarly exposed to various toxic agents never exhibited paired helical filaments.

The observations described here suggest at least three possibilities. The Alzheimer donor brain may have contained metabolic products, possibly associated with the degeneration, which are capable of assembling neurofilaments into paired, helical structures. Such molecules may be postulated to have high affinities for specific sites on normal neurofilaments, resulting in their cross bonding and twisting into a helical configuration. Alternatively, the assembly factor may arise from a viral product present in brain affected by Alzheimer's disease, or, a viral-like agent may have been transferred to the cultured cells and the

paired helical filaments may have resulted from the replication of a transmissible agent in the cultured human brain cells. Although several morphologically distinct types of viral particles were observed in the cultures exposed to the extract from Alzheimer-affected brain their relationship to the paired helical filaments is not yet known.

This research was supported by the Ontario Mental Health Foundation. We thank A. Rodriguez for technical support and Dr J. Deck, Department of Pathology.

Note added in proof: Since the introduction of Alzheimer material into the incubator, repetition of the above experiments has revealed the presence of an occasional control explant with paired helical filaments. Since all other variables have remained unchanged, we interpret this as support for the possibility that paired helical filaments are a response of human neurones to an infectious agent.

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Received 11 October; accepted 12 December 1977.

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Identification of the binding protein which may be the target of penicillin action in *Bacillus megaterium*

PENICILLINS and cephalosporins (β -lactam antibiotics) are believed to kill bacteria by inhibiting reactions involved in the final stages of cell wall assembly¹. It is widely assumed that inhibition of a transpeptidase involved in the insertion of nascent peptidoglycan strands into the cell wall directly leads to cell death. One approach to the study of the lethal action of penicillin has involved the investigation of penicillin-binding proteins (PBPs) which, with few exceptions, are located exclusively in the cytoplasmic membrane of bacteria^{2–4}. PBPs form covalent complexes with benzyl-(¹⁴C)-penicillin, and can be detected by fluorography after sodium dodecyl sulphate (SDS) polyacrylamide gel electrophoresis. All species of bacteria examined contain multiple PBPs. We have shown that the isolated membranes of *Bacillus megaterium* contain five proteins which bind benzylpenicillin covalently and we have measured their relative affinities for the antibiotic⁴. However, the accessibility of the PBPs and their affinities for penicillin may be different in whole cells, and to identify the PBP which represents the lethal target for benzylpenicillin, it is necessary to investigate the binding of the antibiotic to the PBPs of whole cells. We report here that only one of the five PBPs in *B. megaterium* reacts with benzylpenicillin to any significant extent at concentrations just sufficient to kill the cells, and that the characteristics of the inhibition and of the breakdown of the PBP–penicillin complex are consistent with its being the protein which catalyses transpeptidation.

It is difficult to run quantities of whole cell protein sufficient to reveal the PBPs by fluorography in a reasonable time, without overloading the polyacrylamide gel. Because all the PBPs of *B. megaterium* are located in the membrane, it was possible to carry out the experiment by incubating intact cells with penicillin followed by preparation of the membrane fraction and examination of the PBPs. A preliminary investigation showed that release of bound penicillin from the PBPs was slow, with PBPs 1 and 5 having the shortest half-lives of breakdown at

Fig. 3 Electron micrographs comparing paired, helical filaments from an Alzheimer affected brain (a, b) to a paired, helical filament in a cultured foetal brain cell; 35-d *in vitro*. In (a), (c), scale bar 0.1 μ m; b, 0.05 μ m.



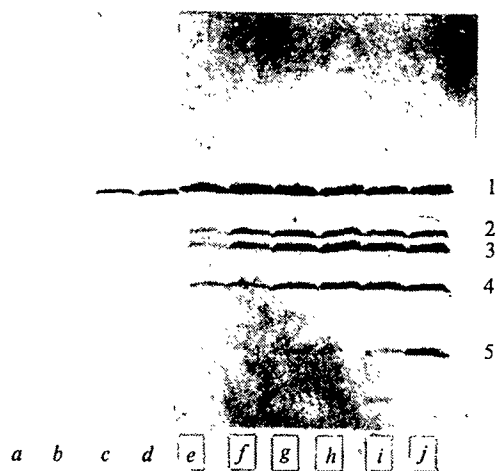


Fig. 1 Fluorogram demonstrating the sensitivity of membrane-bound penicillin-binding proteins in whole cells of *Bacillus megaterium*. 10-ml cultures of *B. megaterium* KM growing exponentially in a complex medium were treated with a range of concentrations of benzyl- ^{14}C -penicillin (51 Ci mol^{-1}) when the cell density had reached $0.3 \text{ mg dry weight ml}^{-1}$. After 5 min at 37°C , 10 mM nonradioactive benzylpenicillin was added, the cells were collected rapidly by centrifugation at 4°C ($27,000g$, 1 min), washed in ice-cold 25 mM Tris-HCl, 5 mM MgCl_2 , $\text{pH } 7.2$, resuspended in 0.3 ml of the same buffer containing lysozyme ($250 \text{ } \mu\text{g ml}^{-1}$) and DNA-ase ($5 \text{ } \mu\text{g ml}^{-1}$), and incubated at 37°C until lysis was complete (5 min). The membranes were collected by centrifugation at $40,000g$ for 20 min and dissolved by boiling for 1 min in $50 \text{ } \mu\text{l}$ buffer containing 10% glycerol, 1% SDS, 1% 2-mercaptoethanol and 0.002% bromophenol blue in 10 mM Tris-HCl $\text{pH } 7.2$. The membrane proteins were separated by electrophoresis on a 10% polyacrylamide slab gel containing SDS (ref. 5), and the penicillin-binding proteins (PBPs 1-5, numbered in order of decreasing molecular weight, ref. 4) revealed by fluorography 6,7 . The following concentrations of benzyl- ^{14}C -penicillin were employed ($\mu\text{g ml}^{-1}$): a, 0.003; b, 0.006; c, 0.01; d, 0.03; e, 0.06; f, 0.1; g, 0.3; h, 0.6; i, 1.0; j, 5.0.

37°C (15–40 min). The conditions of membrane isolation (5 min at 37°C with lysozyme, followed by the remainder of the manipulations being carried out at 4°C (approximately 45 min)) were such that they would not result in significant breakdown of PBP-penicillin complexes.

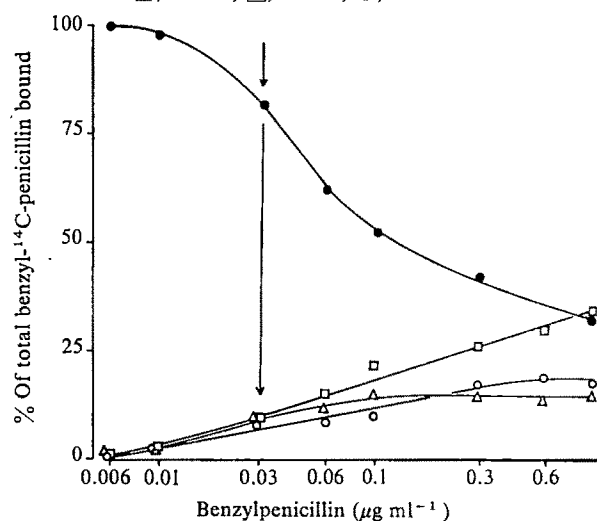
Whole cells of *B. megaterium* were incubated with a range of concentrations of benzyl- ^{14}C -penicillin, collected, washed, lysed by treatment with lysozyme and the protoplast membranes obtained by centrifugation. The proteins of the membrane were fractionated by SDS-polyacrylamide slab-gel electrophoresis 5 and the PBPs detected by fluorography 6,7 . This enabled the affinities of the PBPs for benzylpenicillin in whole cells to be determined. The relative affinities of the PBPs were the same as had been found previously when isolated membranes were treated with benzylpenicillin 4 , although the absolute concentrations of the antibiotic resulting in 50% saturation of the PBPs were lower than when membranes were used. The PBPs are likely to be more accessible in membranes than in whole cells, and this suggests that the amount of penicillin available to react with the PBPs of membranes may have been a limiting factor at the lower concentrations of the antibiotic. This possibility will be considered elsewhere. It is clear from the results in Fig. 1 that PBP 1 has the highest affinity for benzylpenicillin, but this information alone is insufficient to implicate this protein as the lethal target of penicillin. It is necessary to investigate which PBPs bind penicillin when growing cells are labelled at concentrations of the antibiotic which are just sufficient to interfere with growth or cause cell death. The growth of *B. megaterium* as measured by increase in turbidity was inhibited by 50% at a concentration of $0.03 \text{ } \mu\text{g}$ benzylpenicillin per ml; at this concentration, the saturation of PBPs 1–5 in whole cells was 75, 12, 8, 4 and 0% respectively. Over a long period, the inhibition of an essential enzyme by as little as 10% might be sufficient to impair growth, but in this short term growth experiment over a period of three generations,

it seems unlikely that such a degree of inhibition (of PBPs 2 and 3) would account for the immediate inhibition of growth. We therefore conclude that PBP 1 is most likely to be the lethal target of penicillin.

At the minimum growth inhibitory concentration of $0.03 \text{ } \mu\text{g ml}^{-1}$, more than 80% of the total radioactivity bound to the PBPs was present in PBP 1, with approximately 5% on PBPs 2, 3 and 4 (Fig. 2). As the concentration of benzylpenicillin was lowered further to that which inhibited the growth of cells from a small inoculum ($10^6 \text{ cells ml}^{-1}$) in liquid medium ($0.01 \text{ } \mu\text{g ml}^{-1}$), or that which inhibited the growth of single cells on agar ($0.006 \text{ } \mu\text{g ml}^{-1}$), no radioactivity was found associated with PBPs 2–5, while appreciable binding of benzyl- ^{14}C -penicillin to the PBP 1 of intact cells was still demonstrable (Fig. 1). This is further indication that PBP 1 is of prime importance in the interaction of benzylpenicillin with whole cells of *B. megaterium*.

It is possible that benzylpenicillin interacts non-covalently with the killing site, or forms a covalent complex which breaks down so rapidly before or during SDS gel electrophoresis that it is not subsequently detected as a PBP. Under these circumstances it would obviously not be possible to correlate the killing site of penicillin with a particular PBP. The characteristics of the interaction of benzylpenicillin with PBP 1, however, are identical to those of the inhibition of the natural peptidoglycan transpeptidation reaction which is catalysed by wall/membrane preparations of *B. megaterium*. This transpeptidation reaction is inhibited by 50% at $0.1 \text{ } \mu\text{g}$ benzylpenicillin per ml (ref. 8), a concentration which results in approximately 50% saturation of PBP 1 in isolated membranes. Preliminary studies on the interactions of benzylpenicillin with the natural transpeptidation system suggest that a covalent complex is formed, and subsequently breaks down, with a half life of 50–60 min at 23°C . PBP 1 interacts with benzylpenicillin to form a covalent complex which also breaks down with a half life of 60 min at 23°C , and this is consistent with the hypothesis that the natural transpeptidation reaction which represents the lethal target of penicillin action is catalysed by PBP 1. Proof of the hypothesis will have to await the demonstration that purified PBP 1 can catalyse a transpeptidation reaction. PBP 1 has been solubilised and purified almost to

Fig. 2 Binding of benzyl- ^{14}C -penicillin to the proteins of whole cells of *Bacillus megaterium*. The degree of blackening of the X-ray film resulting from the ^{14}C -penicillin-protein complexes was determined by scanning the fluorogram shown in Fig. 1 with a Joyce-Loebl micro-densitometer and determining the areas of the peaks thus obtained by weighing. The amount of radioactivity bound to the individual PBPs at the various concentrations of benzyl- ^{14}C -penicillin is expressed as % of the total radioactivity bound at that concentration. The arrow denotes the concentration of benzylpenicillin which just inhibits growth of the organism over a period of three generations (minimum growth inhibitory concentration). ●, PBP 1; △, PBP 2; □, PBP 3; ○, PBP 4.



homogeneity but no enzymic activity in peptidoglycan biosynthesis has yet been ascribed to it⁴. It will be necessary to devise natural model transpeptidation reactions that can be catalysed by the penicillin-sensitive enzymes involved in the terminal stages of peptidoglycan biosynthesis. Such reactions exist for *Salmonella typhimurium*, in which it has been shown that PBP 4 and probably PBP 1 catalyse transpeptidation⁹, and also for *Escherichia coli*¹⁰, but all attempts to develop similar systems for the Gram-positive bacilli have been unsuccessful.

H.A.C. and S.T.S. acknowledge receipt of research studentships from the MRC.

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Substrate induction of conjugative activity of *Agrobacterium tumefaciens* Ti plasmids

CONJUGATIVE plasmids of bacteria are extrachromosomal genetic elements able to bring about DNA transfer by conjugation. The best-studied system is that of F-like plasmids in *Escherichia coli*, where at least 12 genes are involved¹. Expression of the transfer system in this case is subject to negative control^{2,3}. We report here that the newly discovered conjugative activity of Ti plasmids is inducible by specific amino acid derivatives that are also the substrates for plasmid-coded catabolic enzymes. This finding further extends the analogy between the regulation of the lactose operon and of transfer functions.

The Ti plasmids confer oncogenicity to *Agrobacterium tumefaciens* strains responsible for the crown-gall disease of plants^{4–7}. The plasmids apparently fall into three classes, between which there is only a limited degree of homology^{7–11}. First, octopine Ti plasmids trigger octopine (N-2-(D-1-carboxyethyl)-L-arginine) synthesis in transformed plant cells. Such plasmids also confer to these bacteria the ability to utilise octopine as sole carbon and/or nitrogen sources. All the octopine Ti plasmids isolated thus far from different strains seem to be nearly, if not completely, identical^{7–11}. Second, nopaline Ti plasmids trigger nopaline (N-2-(D-1-3-dicarboxypropyl)-L-arginine) synthesis in transformed plant cells. They also confer to their bacterial hosts the ability to use nopaline as sole carbon and/or nitrogen sources. The nopaline Ti plasmids isolated from different strains show a wider range of relatedness^{7–11}. The third kind of Ti plasmids has, so far, no recognised property other than oncogenicity.

We have shown that the Ti plasmids are conjugative^{12,13} as they can be efficiently transferred to non-oncogenic recipient strains of *Agrobacterium* in certain conditions. In our previous work we made use of the fact that these plasmids are catabolic plasmids to select for transconjugants. We noted that plasmid transfer could only be observed if the crosses had been performed in the presence of the arginine derivative—an 'opine'—that is specific for the type of Ti plasmid used in the transfer experiment. One possible interpretation for this result was that octopine or nopaline

induces the conjugative activity of octopine Ti plasmids and nopaline Ti plasmids respectively. However, as the mixed donor and recipient cultures had been inoculated on media in which the opine was either the source of nitrogen or the source of both carbon and nitrogen, some selection must also have been operating to favour transconjugants relative to recipient cells. The experiments described here were designed to distinguish between the models of induction of transfer and selection of transconjugants.

The purpose of a first series of experiments was to determine whether incubation of the donor cells in an opine-containing medium before mixing with the receptor resulted in the induction of conjugative activity. Octopine was found to induce conjugative activity of the Ti plasmid of strain B6S3 (Table 1). Crosses performed with donors that had been previously cultured on octopine yielded transconjugants whereas donors cultivated on arginine and pyruvate (the immediate products of octopine catabolism¹⁴) did not transfer their plasmid in the absence of octopine. When the filters with the mating mixture were incubated on arginine and pyruvate medium, relatively low frequencies of transconjugants were obtained; however, when octopine was used instead of arginine and pyruvate in the mating medium, high frequencies of transconjugants were observed. Their increased frequency is probably due to the duration of the incubation of the mating mixture (48 h) in the presence of octopine; this probably resulted in an increased number of donors transferring their Ti plasmid and also an enrichment of transconjugants relative to the recipients, since octopine was the sole carbon and nitrogen source in the mating medium. Two other opines, lysopine and octopinic acid, which are synthesised concomitantly with octopine in crown-gall cells¹⁵ and can also be utilised as both carbon and nitrogen source by *Agrobacterium* strains harbouring an octopine Ti plasmid, also induce conjugation, but to a lesser degree than octopine. In contrast histopine, a novel

Table 1 Induction of plasmid transfer

Mating medium	Preculture medium (C and N sources)	
	Arginine + pyruvate*	Octopine
Arginine + pyruvate	$\leq 10^{-7}$	7×10^{-5}
Octopine	2×10^{-3}	8×10^{-3}

Values shown represent transconjugant frequencies amongst the recipients. The donor strain B6S3, which carries an octopine Ti plasmid, was crossed with the recipient strain C58Cl. Single colony isolates of B6S3 were grown in liquid minimal medium containing KH_2PO_4 $8.0 \times 10^{-2}\text{M}$, $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ $6.5 \times 10^{-4}\text{M}$, $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$ $7.0 \times 10^{-5}\text{M}$, $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ $1.7 \times 10^{-6}\text{M}$, $\text{MnCl}_2 \cdot 4\text{H}_2\text{O}$ $1.0 \times 10^{-5}\text{M}$, pH adjusted to 7.0 with KOH 6N, supplemented with either octopine 10 mM or arginine 10 mM plus pyruvate 10 mM as carbon and nitrogen sources. The cultures, in exponential phase of growth, were mixed in a ratio of 10:1 with recipient C58Cl cells exponentially growing on arginine plus pyruvate minimal medium. The bacterial mixtures were diluted to A_{600} of 0.2 and 5-ml samples were forced through Millipore filters (pore size 0.45 μm). The filters were incubated for 48 h at 25 °C on agar (1.5%) minimal medium (mating medium) supplemented with 10 mM octopine, or arginine plus pyruvate. The filters were introduced into test tubes containing 5 ml of sterile water and the bacteria on the filters were suspended by vortex action. These suspensions were then diluted and plated on a selective medium containing octopine 10 mM, rifampicin 50 $\mu\text{g ml}^{-1}$ and streptomycin 500 $\mu\text{g ml}^{-1}$. This medium allows only a very limited growth of the recipients which are resistant to the antibiotics but cannot use octopine as a growth substrate. The donors are eliminated by the action of the antibiotics. The transconjugants were selected for by their capacity to utilise octopine and develop into normal colonies. The plates were scored after incubation for 8 d at 25 °C.

* Replacement of arginine plus pyruvate by glucose (2 g l^{-1}) gave almost identical results.



Fig. 3 Fluorography of SDS-polyacrylamide gel electrophoresis of proteins from oocytes injected with native His mRNA (500 $\mu\text{g ml}^{-1}$) supplemented with commercial poly (A) (500 $\mu\text{g ml}^{-1}$). Fifty oocytes from a single *Xenopus laevis* female were injected with native His mRNA supplemented with commercial poly (A). At different times after microinjection, sets of ten oocytes were incubated at 19 °C in Barth medium containing 1 mCi ml^{-1} of ^3H lysine (67 Ci mmol^{-1}). At the end of the incubations, oocytes were processed as described at Fig. 1 for gel electrophoresis. *a-d*, proteins from oocytes injected with native His mRNA + commercial poly (A); *e, f*, proteins from control oocytes. Incubation periods; *a, e*, 0–4 h, *b*, 4–20 h, *c*, 20–48 h, *d, f*, 48–60 h.

In complete agreement with the previous experiment, it was found that, in oocytes injected with native His mRNA, the rate of synthesis of H_3 , H_{2a} , H_{2b} and H_4 histones increases during the first 7 h, then decreases during the 7–19 h incubation period and becomes finally hardly detectable 43 h after microinjection (Fig. 2). In the case of polyadenylated His mRNA, however, the synthesis rate of the four histones increases up to 19 h and is still very high more than 43 h after microinjection (Fig. 2). One may thus conclude that the addition of a poly (A) segment at the 3'-OH end of the four naturally poly (A)-free mRNAs coding for H_3 , H_{2a} , H_{2b} and H_4 histones functionally stabilises these messages.

We wanted to see whether poly (A) molecules injected at the same time as His mRNA would have the same effect on the stability of histone mRNAs as terminal polyadenylation. So, we compared, in the same kind of experiment as those described above, the functional stability of His mRNA injected alone (at 500 $\mu\text{g ml}^{-1}$) or with commercial poly (A) (100–200 nucleotides long) at 500 $\mu\text{g ml}^{-1}$. Figure 3 shows that the presence of poly (A) injected at the same time as the His mRNA fraction does not increase the functional stability of histone mRNAs, although the concentration of poly (A) is approximately ten times higher than when linked to the 3'-OH end of the message molecules, as in the preceding experiment. It thus seems that, in oocytes, the 3'-OH poly (A) segment does not protect the mRNA molecule simply by competitive inhibition of ribonuclease activity as might be inferred by the work of others in *in vitro* experiments¹³. The protection mechanism must be more specific, and we have indeed shown that the degradation of a poly (A)-lacking mRNA is associated with its translation³.

We suggest that the presence of a poly (A) stretch long enough at the 3'-OH end of four different histone mRNAs ensures their functional stability when injected into *Xenopus* oocytes. These results extend our previous conclusion about the stabilising role of poly (A) obtained from experiments using native and deadenylated globin mRNA^{1–3,5,6} (for review see ref. 14). Preliminary results from experiments using sea urchin embryo histone mRNAs confirm our conclusion.

We thank Professor H. Chantrenne for helpful discussions. This work was supported by a grant from the Fonds Cancérologique de la Caisse Générale d'Épargne et de Retraite and

the State contract Actions Concertées. G.H. and G.M. are Chercheurs Qualifiés of the Belgian Fonds National de la Recherche Scientifique. Part of this work was supported by a grant from the Deutsche Forschungsgemeinschaft to D.G.

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Received 21 October; accepted 23 November 1977.

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Role of the G segment in the growth of phage Mu

USING the single-burst technique we have been able to show that close to one-half of the cells in a lysogenic culture of phage Mu yield no viable phage after induction. Our experiments were prompted by an intriguing observation concerning the G segment of Mu, a region of 3,000 base pairs found in either orientation in both mature phages and prophages^{1,2}; it is thought that G inversions are mediated by inverted repeats of approximately 50 base pairs known to be located at the two ends of G (ref. 3). It was observed that progeny phage derived from the induction of lysogenic cultures contain approximately equal numbers of particles with the G segment in either orientation, but virtually all progeny phage derived from lytic cycles of infection have their G segment in one particular orientation⁴.

The simplest proposal to explain this behaviour is based on the following assumptions⁵: (1) inversion of the G segment occurs only in the prophage state. (2) On induction individual lysogenic cells produce bursts in which all phages have the G segment in the same orientation as the parental prophage. (3) Phages with the G segment in one orientation, say G(+), are viable in lytic infection, with the G(–) orientation they are non-viable. (4) The frequency of inversion of G during growth of a Mu lysogen is such that in any culture of 10^8 cells (the minimum required for electron microscope experiments) the G(+)/G(–) ratio is close to 1.

These assumptions lead directly to the prediction that only one-half of the individual cells in a lysogenic Mu culture yield viable phage after induction.

Table 1 Comparison between tubes tested immediately after inoculation

Tubes per set	Tubes containing bacteria (set 1)	Tubes yielding viable phage (set 2)	Expected tubes* from theory yielding viable phage
132	32	20	17
156	62	30	35

The bacterial strain used was a derivative of the *Escherichia coli* K12 strain CSH55 (ref. 10), CSH55Mu^R/F⁺pro⁺lac::Muets62. To test for the presence of viable phage 0.25 ml of a stationary culture of the indicator bacteria CR63 was added to each tube and these were then incubated overnight at 32 °C. Next day samples from the tubes were spotted onto plates seeded with CR63, and these plates incubated overnight at 37 °C.

*This number makes allowance for the fraction of tubes receiving more than one cell at the multiplicities used.

We set out to test this prediction in experiments reported here, using an adaptation of the classical 'single-burst' experiment of Burnet⁶. In the first experiments a culture of of a mono-lysogenic bacterial strain containing the heat-inducible prophage Muets62 was grown in broth at 32 °C to 10⁸ cells ml⁻¹. The culture was then diluted appropriately and aliquots distributed amongst two sets of broth-containing tubes with such a multiplicity of bacteria per tube (0.2–0.5) that the majority of tubes which received bacteria were inoculated with a single cell. One set of tubes was then incubated overnight at 32 °C and examined next day to determine the number of tubes containing bacteria; the second set was immediately placed at 43 °C for 60 min (conditions which provoke induction and lysis) and each tube then tested for the presence of viable phage.

Table 1 represents the results of two experiments of this type. The ratio between the numbers of tubes yielding viable phage in one set of tubes and those containing bacteria in the parallel set is close to that predicted by the model. However, a drawback of this kind of experiment is that the numbers involved in the comparison between the two sets of tubes are inevitably small and so possible statistical fluctuations are considerable. To obtain more compelling evidence concerning the basic assumption that the induction of individual lysogenic cells often does not yield viable Mu phage, we modified the experimental procedure to obviate the statistical criticism of the previous results.

The starting point of the modified experiments was similar to the previous ones, but the sets of tubes were now left after inoculation at 32 °C before testing, to allow clones of bacteria to develop in those tubes receiving cells. After various periods of time a set of tubes was removed from 32 °C and the contents of each tube (1 ml) split into two equal volumes. One of these was then assayed to determine the number of cells it contained; the other was induced and then tested for the presence of viable phage.

Table 2 Tests of individual clones for viable phage

Tubes per set	156	
Time of incubation (h)	3	4
No. of tubes containing clones	64	60
Average clone size (no. of bacteria)	28	58
Clones yielding viable phage	46	48
Clones yielding no viable phage	18	12
Fraction of clones yielding no viable phage	0.28	0.20

Bacterial assays were performed by adding 0.5 ml of 1.2% molten Difco agar to each 0.5 ml volume to be tested, shaking the tubes, and then incubating them for 2 d at 30 °C. At this stage spherical colonies could easily be seen and counted. Testing of the other 0.5 ml volumes for viable phage was carried out as in the previous experiment.

Table 2 presents the result of an experiment of this type in which the clones were allowed to develop for 3 or 4 h before testing, at which times they contained an average of 28 and 58 cells respectively. It can be seen that even after 4 h growth there were 12 tubes which, after division, yielded bacteria but no viable phage. The chance that this lack of viable phage was due to the absence of bacteria in the induced volumes is negligible, as in 10 of the 12 tubes the bacterial assays on equivalent volumes were greater than 20. This evidence therefore shows quite clearly that some lysogenic Mu cells yield no viable phage on induction.

Also from Table 2 it can be seen that the fraction of clones yielding no viable phage decreases with increasing time, a result interpreted from the theory as reflecting the occurrence of G inversions during bacterial growth. Using methods similar to those for calculating mutation rates⁷ it is possible to estimate from the data that the rate of G inversions per cell per generation is close to 0.03, and that the G(+)/G(–) ratio in a culture of 10⁸ bacteria derived from a single cell would be within 5% of unity.

Our data therefore confirm the predictions of the G inversion model. They are also consistent with a less restrictive scheme which allows a small amount of inversion to occur during vegetative phage growth. By themselves, however, the data do not prove that our observations are related to the inversion of G. The correlation comes from experiments of a quite different kind performed by Kamp *et al.*⁸ These authors have isolated two types of Mu mutant which are inversion-defective. One has a deletion covering the right end of the G segment, while the other is presumed to be defective in the production of the inversion enzyme. In these mutants, with G locked in its G(–) orientation, mature phage are produced from induced lysogens, but these phage are non-viable in lytic infection.

Taken together these two sets of results provide strong evidence that in a normal lysogenic culture the cells with the G segment of the prophage in the G(–) orientation produce non-viable phage particles. Presumably the G segment contains, at least in part, some gene whose function is essential in the lytic cycle of Mu; and in one orientation this gene is non-functional. There are two possible roles for this gene. It could be involved in a step which must occur early in the lytic cycle of Mu if phage production is to take place, but is not needed when a prophage is induced, or it could affect the structure of the phage coat so that the Mu DNA is not able successfully to penetrate host cells. In both cases non-viable phage with the G(–) orientation would arise from infection and lysogenisation with a G(+) particle followed by a G(+) to G(–) inversion in the prophage state, and finally by induction. The results of Bukhari and Ambrosio⁹ suggest that the second of these alternatives is correct.

A.C. is supported by CAPES (Brazil).

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Received 26 August; accepted 16 December 1977.

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The invertible segment of bacteriophage Mu DNA determines the adsorption properties of Mu particles

INTEREST in bacteriophage Mu stems from the highly promiscuous insertion of its DNA into the genome of its host bacterium *Escherichia coli* (see ref. 1 for review). There are two characteristic features of Mu DNA: first, mature Mu DNA contains heterogeneous host sequences at both ends^{2,3}, and second, near the right end, the S end, of Mu DNA there is a 3,000 base pair sequence that can undergo inversion. The structure of Mu DNA

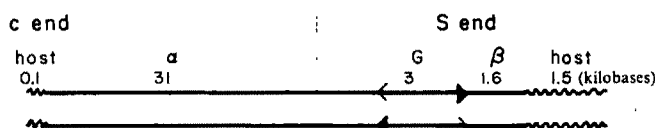
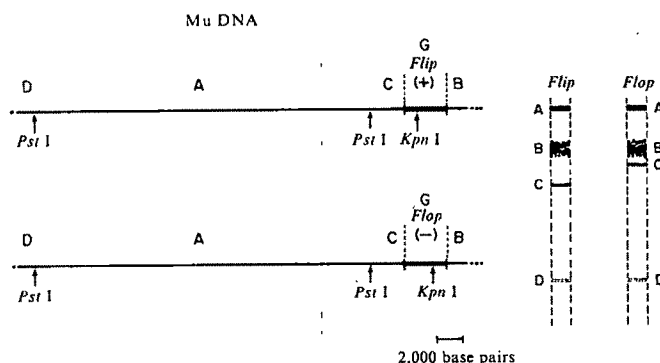


Fig. 1 Structure of Mu DNA. The different segments of Mu DNA are shown in kilobases. The values for the heterogeneous host sequences at the ends are approximate. When two Mu-DNA molecules carrying G in opposite orientations are denatured and reannealed, the G segment can be seen in the electron microscope as a symmetrical non-renaturable bubble. Less than 1% of the renatured molecules show the bubble if Mu particles are grown by infection of sensitive cells. When Mu is grown by induction of a lysogen approximately 50% of the renatured molecules have the G bubble.

is diagrammed in Fig. 1. The invertible sequence, called the G segment, is remarkable in that it is also found in bacteriophage P1 (ref. 4). The inversion of the G segment in Mu occurs in the prophage state and is independent of the *recA* function of the host⁵. According to Hsu and Davidson⁶, the inversion occurs by recombination between identical but inverted sequences of about 50 base pairs flanking the G segment. Allet and Bukhari⁷ have presented evidence that a Mu function, located within or close to the G segment, is required for the inversion reaction. When Mu particles are grown by induction of a lysogen, about half of the particles contain DNA with one orientation (referred to as the + or *flip* orientation) and the rest of the particles have the G segment in the reverse orientation (the - or *flop* orientation). However, when Mu particles are grown by infection,

Fig. 2 Scheme for identifying the G orientation of Mu DNA. Phage DNA is indicated by solid lines and host DNA by dotted lines. The arrows represent cuts made by restriction endonucleases *PstI* and *KpnI* on Mu DNA in both the *flip* (+) and *flop* (-) orientations. The resulting fragments A, B, C and D give patterns, after electrophoresis in agarose gels, as depicted on the right. Fragments B and D are seen as fuzzy bands because they are linked to host sequences of variable lengths. The size of fragment C, spanning the left region of G and the sequence immediately adjacent to G, depends upon the orientation of G (see the right two columns of Fig. 3).



almost all of the particles contain DNA with the *flip* orientation. The predominance of the *flip* orientation is obtained even if the phage lysate used for infection contained equal numbers of the *flip* and the *flop* orientations. We show here that the predominance of the *flip* orientation after infection results from the inability of the *flop* containing particles to adsorb properly to the bacterial cells.

An important step in explaining the apparent dichotomy of G inversion was made by Chow, Kamp and Kahmann who isolated a mutant of Mu in which the flip-flop reaction of G did not occur⁸. Kamp *et al.* showed that this G inversion (*gin*⁻) mutant produced plaques only when the G segment

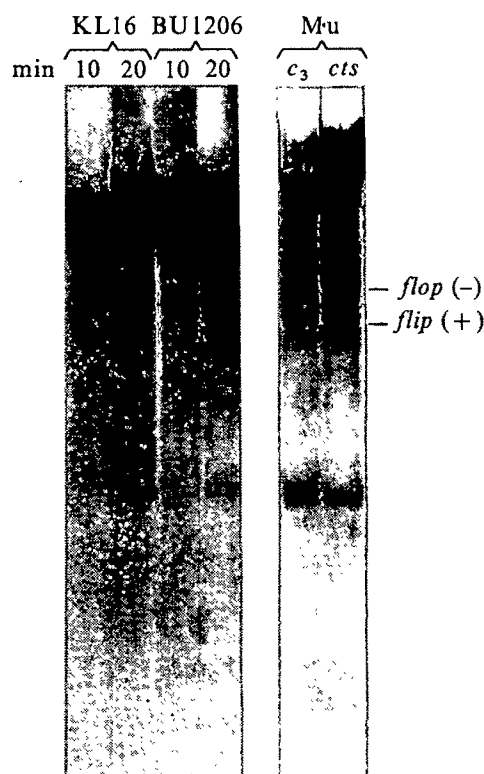


Fig. 3 Infection of *E. coli* by ³²P-labelled Mu particles. ³²P-labelled Mu preparation was obtained by induction of strain BUHM8305 (containing the temperature-inducible mutant Mu *cts* 62) and contained both the *flip* and the *flop* particles. *E. coli* K12 strains used for infection were KL16, an Hfr prototrophic strain, and BU1206, a strain carrying the plasmid pRM101. The plasmid pRM101 contains the G segment of phage P1 and apparently has the necessary functions to carry out G inversion. (The plasmid was obtained from D. Vapnek, University of Georgia.) Logarithmically growing cells were centrifuged and resuspended in Mu buffer¹¹. The phage was adsorbed for 15 min at room temperature and the mixture was then diluted 10-fold into prewarmed LB broth at 37 °C containing 2.5 mM Mg²⁺ and 1 mM Ca²⁺ (ref. 11). The mixture was gently shaken at 37 °C. At 10 and 20 min after mixing, the mixture was centrifuged. The cells were washed three times with cold Tris (0.01 M)-EDTA (0.001 M) buffer pH 8.0 and the DNA was phenol extracted. The DNA samples were digested with *KpnI* and *PstI*, the digests were fractionated in 1% agarose gels by electrophoresis and the gels were autoradiographed. The two columns at the right show control Mu DNA preparations (not radioactively labelled), digested with *KpnI* and *PstI*. Mu *cts* represents the standard Mu *cts* 62 lysate made by induction, showing both the *flip* and the *flop* orientation, used for infection experiments. Mu c3, a clear-plaque mutant of Mu, grown by infection shows only the *flip* orientation. After infection of KL16 and BU1206 with Mu *cts* 62 particles, containing both G orientation, only the lower G band representing the *flip* orientation can be seen.

was in the + (*flip*) orientation⁸. To discover in which way the Mu *flop* particles are defective, we have followed the fate of Mu DNA molecules after infection as well as after induction. In these experiments, the orientation of G in the Mu DNA molecules was determined by cleavage with restriction endonucleases. The principle of such analysis has been enunciated by Allet and Bukhari⁷. We used the enzymes *KpnI* (from *Klebsiella pneumoniae*) and *PstI* (from *Providencia stuartii*) Kahmann *et al.*¹⁰ originally determined the cleavage sites for these enzymes in Mu DNA and found that these enzymes, in combination, are very useful in identifying the G orientation. As shown in Fig. 2, the only *KpnI* cleavage site in Mu DNA is located within G, whereas both *PstI* cuts are outside G, one cut being close to the left of G. Depending on the orientation, a different fragment spanning the left part of G is obtained when Mu DNA is cut with a combination of *KpnI* and *PstI*.

We prepared ³²P-labelled Mu particles, as described by Bukhari and Ljungquist¹¹, and infected *E. coli* cells with these particles. At various times after infection, the cells were centrifuged and the DNA was extracted. The orientation of G in the radioactively labelled Mu DNA molecules was examined by autoradiography after cleavage with *KpnI* and *PstI* and electrophoresis in agarose gels. As shown in Fig. 3, only the *flip* orientation could be detected in the cells. Since the starting

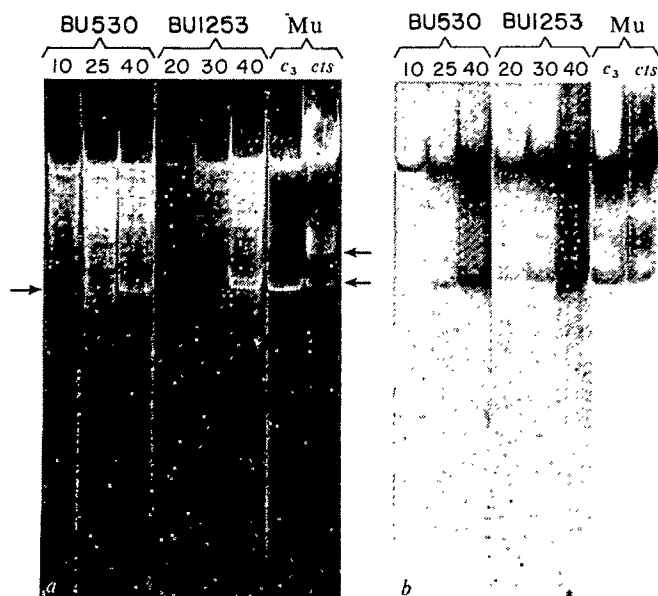


Fig. 4 Orientation of G in replicating Mu DNA after infection. Mu *cts* 62 particles grown by induction were used to infect strain BU530, a prototrophic Mu-sensitive strain of *E. coli* K12, and strain BUI253, an *E. coli* strain carrying the plasmid pRM101, which contains the G segment of phage P1 (see Fig. 3). At various times after infection the cells were washed and the DNA was extracted with phenol. The DNA samples were cut with *KpnI* and *PstI* and fractionated in 1% agarose gels by electrophoresis. The fragments were stained with ethidium bromide and photographed under ultraviolet light. The fragments were then denatured in gels, 'blotted' onto nitrocellulose paper and hybridised with ³²P-labelled Mu DNA^{3,13}. *a*, The ethidium bromide stain photograph is on the left and *b*, the autoradiograph after hybridisation. The right-hand column in both (*a*) and (*b*) shows the Mu *cts* DNA extracted from particles used to infect the cells. This DNA shows both G orientations as compared to Mu *c3* DNA grown by infection, which has only the *flip* orientation. It can be seen in the autoradiograph (*b*), that only the *flip* orientation is detected at different times. That the Mu DNA is replicating is shown by the increase in hybridisation of the Mu fragments. The hybridisation background, particularly visible at 40 min after infection, results from random integration of Mu DNA into the host DNA during growth (see ref. 13). Note that in the ethidium bromide stain at the left the G *flip* fragment is visible at 40 min, indicating a large-scale replication of Mu DNA.

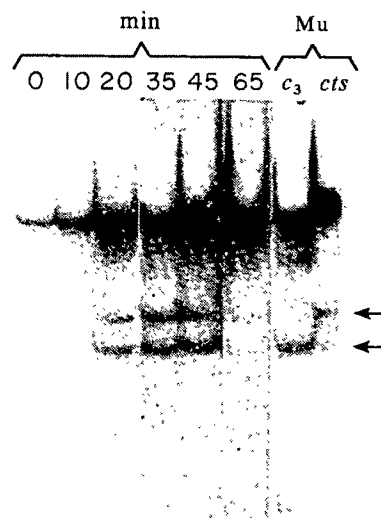


Fig. 5 Orientation of G in replicating Mu DNA after induction. Strain BUHM8305, lysogenic for Mu *cts* 62, was heat-induced by shifting the logarithmically growing culture to 44 °C for 20 min and then shifting to 37 °C. Just before induction and at various times thereafter, samples were withdrawn and DNA was extracted with phenol. The DNA was cut with *KpnI* and *PstI*, fractionated by electrophoresis in 1% agarose gels. The fragments were then denatured and transferred to nitrocellulose paper and hybridised to ³²P-labelled Mu DNA^{3,11}. The figure shows the autoradiograph after hybridisation. The two columns at the right are Mu *cts* (showing both G fragments) and Mu *c3* (showing only the *flip* fragment). Both G orientations are detected in the lysogen before induction. After induction both G fragments increase in amount in parallel. The background hybridisation from 20 min onwards reflects integration of Mu at different sites generating random new fragments that hybridise with Mu DNA¹³. By 65 min the culture has undergone substantial lysis, decreasing the yield of cells and thus the intensity of DNA bands is reduced.

lysate contained both the *flip* and *flop* orientations, this shows that the *flop* particles were not able to infect the cells. Similarly, we found that infected cells contained Mu DNA in the *flip* orientation only whereas the supernatant contained a large population of unadsorbed Mu particles with G in the *flop* orientation. Consistent with this result, we have observed that the Mu phage particles purified after induction of Mu-sensitive host contain proportionally more phage with the *flop* orientation than phage prepared from Mu-resistant host. Presumably, this is because of preferential adsorption of *flip* particles to cell debris during purification.

To determine the orientation of G in the replicating Mu DNA molecules, we either infected *E. coli* cells with unlabelled Mu particles or induced Mu lysogens. The DNA was extracted at various times after infection or induction and cleaved with *KpnI* and *PstI*. The fragments were separated in agarose gels by electrophoresis, denatured and transferred to nitrocellulose paper by the procedure of Southern¹². After hybridisation with ³²P-labelled Mu DNA, the DNA fragments hybridising to Mu DNA (that is, Mu fragments) were visualised by autoradiography, as described previously^{3,13}. Only one orientation of G was detected in replicating Mu DNA, as late as 40 min after

infection (Fig. 4). Even in a strain that contained the G segment of phage μ cloned in a plasmid, and thus had the G inversion system of μ only the *flip* orientation of G could be observed. This shows that the rate of G inversion is slow and that most of the infecting molecules remain in the *flip* configuration throughout the lytic cycle. As shown in Fig. 5, both G orientations were detected in a *Mu cts* lysogen, before or at various times after induction. This result thus provides direct proof that G inversion occurs in lysogenic cells before induction and that, after induction, both orientations replicate equally well. Interestingly, the G orientation of replicating *Mu* DNA can be determined after infection or after induction, even without DNA-DNA hybridisation and autoradiography. This is because as *Mu* replicates and *Mu* DNA copies accumulate, the *Mu* G fragments stand out above the background. (See Fig. 4.) We have used this method to screen for *Mu* mutants defective in G inversion (unpublished).

We conclude from these experiments that the reason why *Mu* particles with only the *flip* (or +) orientation of G are obtained after infection is that the particles with G in the opposite orientation (*flop* or - orientation) fail to infect the host cells. This block in infection seems to result from an inability of *Mu flop* particles to adsorb to the cells. It is not clear whether the *flop* particles do not adsorb at all or whether the adsorption is reversible such that the *flop* particles are easily detached from the cell surface. Apparently, when lysates are prepared by infection there is a selection for the *flip* particles at each infectious cycle. Since the rate of G inversion is slow, only *Mu* particles with the *flip* orientation accumulate in the lysates prepared by infection. These results extend the observation of Kamp *et al.*⁹, on the non-viability of the *flop* particles and complement the results of Symonds and Coelho¹⁴ who have shown that only some of the cells in a culture of a *Mu* lysogen give rise to infectious centres and that the rate of G inversion is slow that is 0.03 per cell per generation.

The inability of the *Mu flop* particles to adsorb properly indicates that these particles lack a phage component needed for adsorption. Evidently, this component is made only when G is in the *flip* orientation. This case provides an interesting example of the *flip-flop* control of gene expression. A similar mechanism has been postulated for phase variation in *Salmonella*¹⁵.

We thank Phyllis Meyers and R. J. Roberts for providing some enzyme preparations and M. S. DuBow and R. Gudor for useful discussions. This work was supported by a grant from the US NSF (PCM 76-13460). A. I. Bukhari holds a Career Development Award from the NIH.

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Received 29 September; accepted 5 December 1977.

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Inversion of the G DNA segment of phage *Mu* controls phage infectivity

THE 3,000 base pair-long G segment of temperate coliphage *Mu* DNA has been shown to invert spontaneously with high frequency^{1,2}. Inversion, which is independent of the *rec* system of the host³, results in *Mu* phage with the G segment in either orientation. Nearly equal numbers of both G orientations are observed in a lysogen or when a lysate is obtained by induction of a lysogen. When phage is grown by a series of infections one and the same G orientation prevails⁴. One attractive explanation for these observations implicates inversion of the G segment in the control of phage or host functions. We report here experiments which show that the orientation of the G segment determines infectivity of *Mu* phage particles and that G inversion is mediated by a phage-specific enzyme system.

In our studies we used a recently isolated plaque-forming substitution mutant, *Mu* 445-5, which no longer inverts its G segment but apparently has an intact G region⁵. We refer to this phenotype as *gin*⁻ for G inversion. The orientation of the G segment in *Mu* 445-5 is the same as that found to predominate after lytic infection. We shall call this orientation G(+) and the opposite orientation G(-). To test possible effects of G orientation on *Mu*, we attempted to invert the G segment of *Mu* 445-5 *in trans* by using a helper phage. We constructed a double lysogen with *Mu* 445-5 on the host chromosome and a *Mu gin*⁺ helper inserted in an episome. This double lysogen was induced and the DNA of the phage progeny was analysed by heteroduplex formation with appropriate reference *Mu* DNA (Fig. 1). The G segment of *Mu* 445-5 phage DNA produced from the double lysogen was found in either orientation, indicating that the *gin*⁻ phenotype of *Mu* 445-5 is recessive and can be complemented by a *gin*⁺ phage. This result shows that at least part of the enzyme system responsible for G inversion is specified by *Mu* itself. Removal of the helper prophage from the double lysogen should result in subsequent dilution of the *gin* enzyme from the cell and thus freeze the G segment of the *Mu* 445-5 prophage in either the G(+) or the G(-) configuration. Strains that had spontaneously lost the episome carrying the *gin*⁺ helper prophage fell into two classes with approximately equal frequency. The first class released normal plaque forming *Mu* 445-5 phage, while the second class lysed after heat induction but produced no plaque forming phage. Nevertheless these lysates contained phage particles which could be banded in caesium chloride density gradients and from which normal size DNA could be isolated. The yield and density of these phage particles were virtually the same as for the plaque forming *Mu* 445-5 phage. Even concentrated lysates did not result in killing or lysogenisation when spotted on a lawn of *Mu*-sensitive bacteria. As determined by restriction enzyme analysis (Fig. 2), the defective particles obtained from several independently isolated lysogens always contained *Mu* 445-5 DNA with the G segment in the G(-) configuration, whereas all plaque producing lysogens yielded *Mu* 445-5 phage with the G segment in the G(+) orientation. The *Mu* 445-5 *gin*⁻ G(-) lysogens were then re-exposed to the *gin* system provided by the episome with the *Mu gin*⁺ helper prophage. As before, removal of the episome resulted in two classes of lysogens—those producing non-infectious *Mu* 445-5 G(-) phage and those yielding infectious *Mu* 445-5 G(+) phage. These results show that the only difference between the defective and non-defective *Mu* 445-5 phage particles is the orientation of their G segments, and that the inability of the *Mu* 445-5 *gin*⁻ G(-)

prophage to produce infectious phage can be reversed by inverting its G segment from the (−) to the (+) orientation. Figure 3 illustrates and summarises these experiments.

Because Mu 445-5 has an insertion (which we have shown to be part of insertion sequence IS2) we could not generalise

this conclusion with absolute certainty. However, indirect evidence suggests that Mu wild-type phage with a G(−) configuration is also defective. It was found that not all cells lysogenic for a temperature-inducible Mu produce phage after heat induction (D. K., unpublished). In view of the

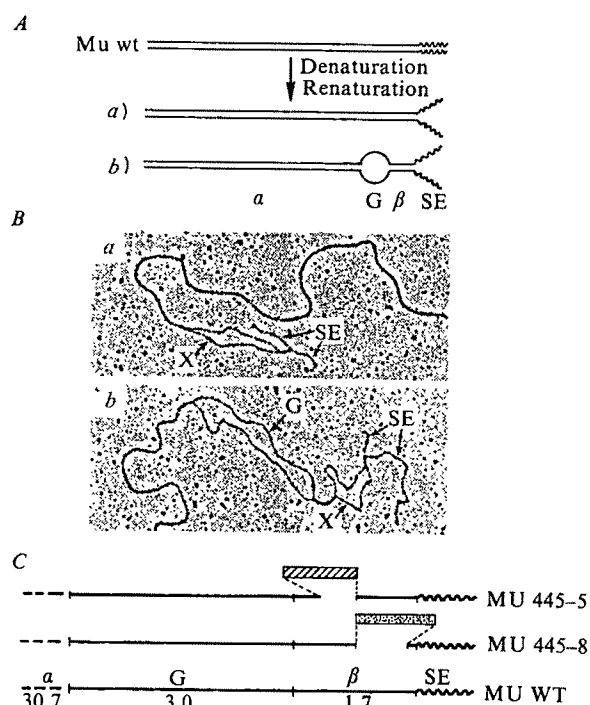


Fig. 1 Determination of the G segment orientation by heteroduplex analysis. *A*, Schematic presentation of DNA structures observed after denaturation and self-annealing of wild type Mu DNA extracted from phage grown by induction⁴. The various segments are designated as alpha, G, beta, and split ends (SE). The lengths of the heterogeneous bacterial DNA comprising the split ends (—) are variable and range from a few hundred to about 3,000 nucleotides. In about half the heteroduplexes (*a*), the G segment is duplex. The single-stranded G loop in the other half (*b*), is due to annealing of Mu strands with G segments in opposite orientations, a result of inversion. *B*, EM heteroduplex determination of the orientations of the G segment in Mu 445-5 grown in the presence of a helper phage. DNA species were denatured with 0.2 M NaOH, neutralised with 2 M Tris-HCl, pH 7.2, and then reannealed at room temperature for 50 min in 50% formamide at a total DNA concentration of 4 μ g ml⁻¹. Renaturation was stopped by dialysis against 0.01 M Tris, 0.001 M EDTA, pH 8.5 at 4°C. Sample grids for electron microscopy were prepared by the formamide variation of the Kleinschmidt technique¹¹⁻¹³. Micrographs were taken with a Philips 201C electron microscope and printed at about the same magnification. Only the right ends of the heteroduplexes are shown. DNA was isolated from phage produced after heat induction of the double lysogen DK850 which is $\Delta pro lac Sm^R$ (Mu cIts62 445-5)/F' *pro lacI::Mu cIts62* Lts (the Mu Lts phage was obtained from A. I. Bukhari¹⁴). The orientation of the G segment of the Mu 445-5 phage was determined from heteroduplexes formed with Mu 445-8. Mu 445-8 is a deletion-substitution mutant in the right half of beta⁵ and was propagated by infection so that its G segment was in the (+) orientation. Mu 445-5 is a deletion substitution mutant in the left half of beta⁵. Heteroduplexes between Mu 445-5 and Mu 445-8 can be distinguished from those of Mu 445-5/Mu or Mu/Mu 445-8 because of their non-overlapping substitution-deletions (see *C*). *B-a*, A heteroduplex of Mu cIts62 445-5/Mu 445-8, showing a duplex G region, a split end and the noncomplementary substitution loop (X) in beta. The G segment of Mu cIts62 445-5 is in the G(+) orientation. *B-b*, A heteroduplex of Mu cIts62 445-5/Mu 445-8 showing the noncomplementary loop in the beta region identical to (*a*), a split end and a G loop, indicating that the G segment of the Mu cIts62 445-5 phage is present in the G(−) configuration. *C*, Diagrammatic map of the G beta region of phages Mu 445-5 and Mu 445-8. Solid lines represent Mu DNA. Deletions are indicated by gaps and substitutions by shaded bars. Sizes of the individual segments are given in kilobase pairs.

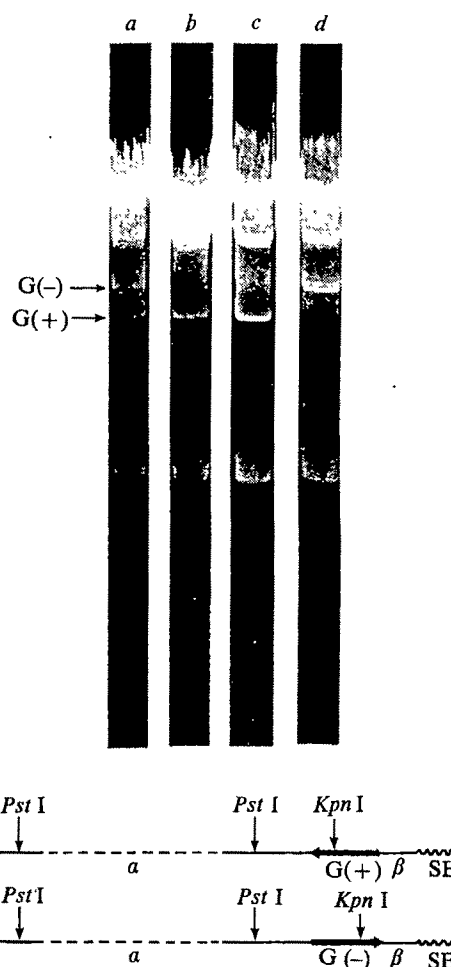


Fig. 2 Determination of the G segment orientation by agarose gel electrophoresis of Mu DNA cleaved with restriction enzymes. The principle of analysing the G segment orientation with restriction enzymes, first described by Allet and Bukhari who used the enzyme *HindIII*⁷, is based on the cleavage of Mu DNA with an enzyme that cleaves the G segment at least at one asymmetrically located site, such that DNA containing G in the (+) orientation produces a restriction fragment of one size while DNA with G in the (−) orientation yields a fragment of different size. Except for Mu *vir*, which was grown by infection, all other phages were propagated by induction. Phage and DNA preparation have been described previously¹⁵. Samples of 2 μ g DNA were digested at 37°C with an appropriate amount of *PstI* and *KpnI* restriction endonucleases in a buffer containing 0.06 M Tris-HCl (pH 7.9), 0.06 M MgCl₂, 0.06 M 2-mercaptoethanol. After digestion, sucrose and bromophenol blue were added to final concentrations of 5% and 0.02% respectively. Slab gel electrophoresis was through 1.2% agarose for 4 h under a constant voltage of 120 V, essentially as described by Sugden *et al.*¹⁶. After staining with ethidium bromide, bands were visualised by excitation with short wave ultraviolet light and photographed through a Kodak no. 23 red filter with Polaroid film P/N55. The *PstI*-*KpnI* fragments of different size are obtained in digests of Mu cIts62 DNA (*a*), because of the presence of both G segment orientations. The smaller of these fragments is characteristic of the orientation found in Mu *vir* (*b*) and in Mu cIts62 445-5 G(+) DNA and is indicated by G(+). The larger fragment is produced when the G segment is in the G(−) configuration, as in Mu cIts62 445-5 G(−) DNA (*d*). It co-migrates with the diffuse right end fragment. The largest band represents the *PstI* fragment generated from the alpha region of the Mu genome. The second largest band represents the *PstI* fragment from the right end, which remained uncleaved by *KpnI* due to low enzyme activity. The smallest fragment originates from the left end of the molecule.

results with Mu 445-, it was tempting to speculate that this phenomenon, found independently by Symonds and Coelho (personal communication), reflects the existence of a sub-population of Mu wild-type lysogens that produces only defective G(-) phage particles on induction. The Mu 445-5 *gin*⁻ lysogens provided the necessary control to test this assertion, because a population of these lysogens is homogeneous with respect to the orientation of the G segment. In fact, every Mu 445-5 G(+) lysogen gives rise to an infectious centre after heat induction. In contrast, for every *gin*⁺ strain tested thus far the frequency of infectious centres may vary from experiment to experiment but never reaches 100% (Fig. 4). Furthermore, this variation seems to reflect the proportion of the prophages which are in the G(+) orientation, because analysis of Mu *gin*⁺ DNAs shows that the ratio of G(+) to G(-) phage also varies. When phage lysates were prepared from the same cultures that had been tested for infectious centres, and DNA from these phages was analysed for the orientation of G segments, we found that the percentage of phage chromosomes with a G(+) orientation was directly proportional to the yield of infectious centres of each culture (Fig. 4). If one assumes that the G segment hardly ever rotates in an induced lysogen, most lysogens would produce either G(+) or G(-) phage. Because there is no significant difference in the yield of G(+) and G(-) phage particles, the ratio of G(+) to G(-) phage in the lysate would then equal the ratio of G(+) to G(-) prophages at the time of heat induction. Thus the yield of infectious centres can be correlated with the ratio of G(+) to G(-) prophages. This implies that Mu *gin*⁺ prophages, which are temporarily in a G(-) configuration when heat induced, produce defective phage particles.

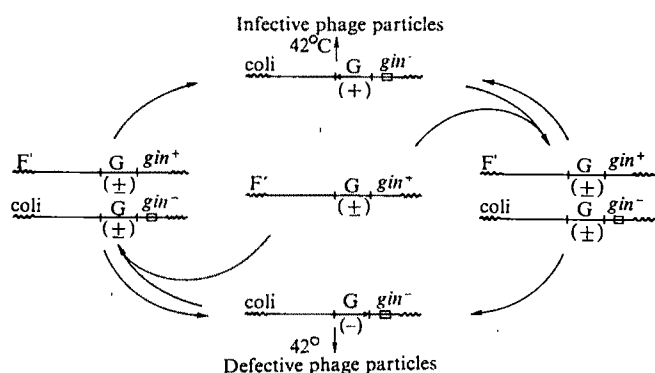


Fig. 3 Inversion of the G segment of Mu 445-5 promoted by a Mu helper prophage. DK984: Δlac pro Sm^R (Mu cIts62 445-5 G(+)) *gin*⁻ was used as a recipient for mating with DK654: Δlac pro Sm^R/F' pro *lacI*:: Mu cIts62 Lts G(+) *gin*⁺. Growth and mating conditions were described by Miller¹⁸. Transconjugants were selected as red colonies (*lac*⁺) on MacConkey lactose streptomycin plates. The double lysogens were grown overnight before they were streaked out on MacConkey lactose plates. Colonies that had been cured of the episome spontaneously occurred with a frequency of about 0.1-1% and were identified as white colonies (*lac*⁻). After restreaking, these colonies were tested for Mu phage production by stabbing them into a lawn of Mu-sensitive indicator bacteria. Thirty to fifty colonies were tested in each step. Three cycles of conversion of G(+) to G(-) and back to G(+) were conducted without any apparent change in the outcome of the experiment. To exclude the possibility that G segments are exchanged by recombination between the episome and chromosome rather than that they are inverted intramolecularly, two control experiments were performed. Rotation of the G segment, G(+)→G(-)→G(+), was not affected by a *recA* mutation of the host. Second, an episome carrying a Mu 445-5 *gin*⁻ G(+) prophage instead of a Mu *gin*⁺ helper was introduced into a strain lysogenic for Mu 445-5 *gin*⁻ G(-) and this double lysogen was subjected to the procedure as described above. Out of 36 colonies that had been cured of the episome, none had acquired a Mu 445-5 *gin*⁻ G(+) prophage.

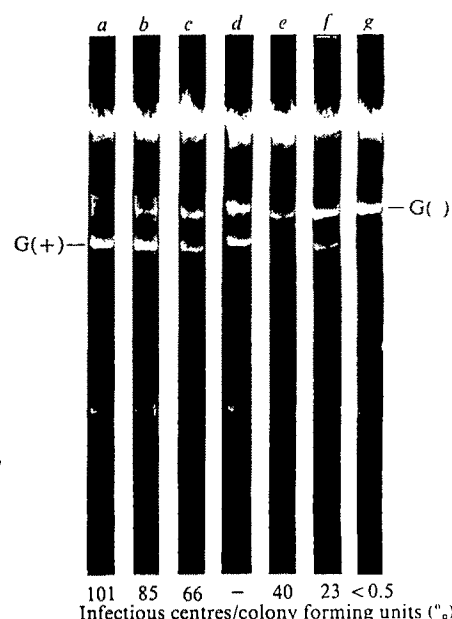


Fig. 4 Infectious centre production of various strains lysogenic for Mu and its correlation to the orientation of the G segment. Analysis of phage DNAs with restriction enzymes *Pst*I and *Kpn*I was as described in Fig. 2. Samples of the lysogenic cultures were taken immediately before heat induction and were diluted in broth to about 1×10^8 colony forming units ml⁻¹. One aliquot was plated at 34 °C to measure the number of cells. In order to determine the number of infectious centres, another aliquot was heat induced at 42 °C for 10 min, mixed with Mu-sensitive indicator bacteria and plated with soft agar at 40 °C. The ratio of infectious centres to colony forming units is given in % of average numbers derived from 7-10 parallel platings. In every experiment, 50-100 colonies from plates used to determine the number of colony forming units were routinely tested for lysogeny and all were found to produce plaque forming phage. This excludes trivial explanations for the lower yield of infectious centres such as frequent spontaneous loss of the prophage or frequent mutation resulting in a defective phage. The results for the following Mu lysogens are shown: a, DK948: (Mu cIts62 445-5 G(+)); b, DK588: (Mu cIts62 *mom*⁻); c, DK392: (Mu cIts62 *cII*31); d, DK1018: (Mu cIts62 445-5 G(-))/F' pro *lacI*:: Mu cIts62 445-5 G(+); e, DK394: (Mu cIts62); f, DK722: (Mu cIts62 *mom*⁻) Mu⁺; g, DK947: (Mu cIts62 445-5 G(-)). The double lysogen in (d) is included as a standard for a 50:50 ratio of G(+) and G(-) molecules. The bacterial hosts are CSH50: Δlac pro Sm^R in (a), (d), (g) and the prototrophic K12 strain W8 in (b), (c), (e) and (f). DK722 was derived from DK588 by selecting for resistance against Mu *vir*. The *mom*⁺ and the *cII*¹⁸ mutations have been described elsewhere. The absolute numbers for infectious centres and the G(+)/G(-) ratios are not reproducible for a given Mu lysogen unless it is *gin*⁻, and the numbers differ from culture to culture within the range that is shown here. Only the correlation between % infectious centres and G(+)/G(-) is consistently found to be the same. The different genetic make-up of the lysogens therefore does not account for the difference in absolute values.

This finding is consistent with our results obtained in *gin*⁻ conditions.

These results have to be reconciled with the current view that all essential functions of phage Mu are located in the alpha region to the left of the G segment⁸. The *mom* function, which is possibly controlled by G inversion, is not essential for Mu growth⁸. Our preliminary findings that Mu 445-5 G(-) phage is deficient for the S gene product raise the possibility that expression of the S function depends on the orientation of the G segment. The S gene, which has been mapped to the immediate left of the G segment³, could in fact be located partially in the G segment and then be split by G inversion. A recent refinement of the correlation between the physical and genetic map at this particular location (M. Howe, personal communication) seems to support this view. An alternative explanation

would be that a promoter is coupled to the S gene only when the G segment is in the (+) orientation. This kind of mechanism has been proposed in the case of *Salmonella* phase variation⁹. Genome rearrangement has also been envisaged as a means of controlling gene expression in eukaryotic cells¹⁰. Therefore the *gin* mediated inversion of the G segment in phage Mu is a useful paradigm for hypothetical control mechanisms that might play a part in the expression of genes in other prokaryotic as well as eukaryotic organisms.

We thank R. J. Roberts and P. A. Myers for providing restriction endonucleases. This work was supported by fellowships of the Deutsche Forschungsgemeinschaft and the Robertson Research Fund to D. K. and R. K., by US NSF grant GB-32041 and NIH grant GM 17612 to D. Z., by NIH grant GM 23996 to L. T. C. and by NIH/NCI Cancer Research Center grant CA 13106 to J. D. Watson.

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Involvement of phage Mu-1 early functions in Mu-mediated chromosomal rearrangements

THE temperate phage Mu-1 not only integrates at any location in the chromosome of its host *Escherichia coli*¹ but also provokes different types of chromosomal aberrations: insertions of extrachromosomal circular DNA^{2–4}, translocations, deletions and inversions of chromosomal segments (refs 5, 6, Howe and Bukhari, unpublished results and M.F., in preparation). Immunity to phage Mu prevents the induction of such events, indicating that Mu gene expression is required for these rearrangements. Furthermore, the direct participation of Mu DNA in the generation of chromosomal aberrations is demonstrated by the observations that one entire Mu genome is always adjacent to the site of the deletions, two entire Mu genomes in the same orientation flank the inserted and translocated DNA fragments, and inverted chromosomal segments are surrounded by two entire Mu genomes in opposite orientations (ref. 22 and M.F., in preparation). Dependence of such Mu-induced alteration on the A and B early phage functions was investigated. *A*[–]

mutants of Mu are unable to mediate all the events tested, while *B*[–] mutants still promote deletion. Based on the results presented here, and on other data, we propose that the A function directly concerns the insertion of the phage and that the B gene codes for a replication function.

The molecular mechanism by which Mu causes chromosomal rearrangements is not known, although all observations made so far are consistent with the idea that the Mu enzymes and the sites on Mu DNA used for normal Mu integration are necessary and sufficient for Mu to mediate these events⁸. All mutants of Mu which result in altered capacity to integrate have been found to affect two of the early functions, either A or B. Such mutants neither replicate nor synthesise late Mu-specific mRNA^{9,10} and on infection of a sensitive host they are unable to mediate integration of extrachromosomal DNA^{2,3}. When present as an induced prophage, *A*[–] mutants still show that defect, but *B*[–] mutants do efficiently mediate integration events⁴. We have studied the capacity of *A*[–] and *B*[–] prophages to mediate deletions and translocation events.

Mu-mediated deletion formation can be readily detected in strains which carry a *Mu*c62 prophage in the *trp* operon (*trp* : : *Mu*c62). After growth at 37 °C, leading to partial induction of the thermoinducible prophage, clones with a deletion in the *tonB*–*trp* region (for revised map of *E. coli*, see ref. 11) may be selected (at 30 °C) for their resistance to phage Φ 80 and the col VB colicins. The frequency at which *tonB* deletions appear in partially induced Mu lysogens is $\sim 10^{-4}$, that is ~ 500 times higher than the spontaneous frequency of 10^{-8} – 10^{-7} at which deletions are formed in that particular region (see Table 1). *sup*⁺ and *sup*[–] strains were isolated which carry either a *Mu*c62 *Aam* or a *Mu*c62 *Bam* or a *Mu*c62 X (*Mu*c62B : : *IS*1 (ref. 12)) prophage in the *trp* operon. These were induced at 37 °C (42 °C in the case of Mu X lysogen) and the frequencies at which *tonB*–*trp* deletions were generated were measured. The frequency of appearance of *tonB* clones

Table 1. Induction of *tonB* deletions by induced *Mu*c62 *A*[–] and *Mu*c62 *B*[–] prophages inserted in the *trp* operon

Induced strain		Frequency of appearance of <i>tonB</i> clones
<i>supF</i> [–] <i>trp</i> : : (<i>Mu</i> c62 <i>Aam</i> 1093)	Positive control	2.6×10^{-5}
<i>supF</i> [–] <i>trp</i> : : (<i>Mu</i> c62 <i>Bam</i> 1066)		4×10^{-5}
<i>sup</i> ⁺ <i>trp</i> : : (<i>Mu</i> c62)		10^{-4}
<i>sup</i> ⁺ <i>trp</i> : : (<i>Mu</i> c62 <i>Bam</i> 1066)		1.1×10^{-4}
<i>trp</i> : : (<i>Mu</i> c62 X3600)		2×10^{-4}
<i>sup</i> ⁺ <i>trp</i> : : (<i>Mu</i> c62 <i>Aam</i> 1093)		1.8×10^{-6}
<i>sup</i> ⁺ (<i>Muc</i> ⁺) <i>trp</i> : : (<i>Mu</i> c62)	Negative control	1.2×10^{-6}
<i>supF</i> [–] <i>trp</i> ⁺		2×10^{-7}

sup⁺ strains are derived from CSH54 (ref. 17) and are Δ *pro-lac*, *his*[–], *thi*[–] str^R. *supF*[–] strains are derived from QD5003 (ref. 18) HfrH *supF*[–]. The *trp* : : (*Mu*c62 X 3600) is derived from HC-B-7-18 Hfr P4X *metB*, *argEC-1*. *Muam* mutations were described by Howe¹⁸. Cultures were grown overnight at 37 °C (42 °C) in L broth, starting from a single colony, without aeration. One loopfull of each culture was resuspended in 5 ml L broth and incubated up to stationary phase without aeration at 37 °C (42 °C). Cultures were titrated on LB plates at 30 °C. 0.1 ml of the 10^{-1} dilutions was mixed with 0.2 ml of Φ 80vir (10^{11} phage ml^{–1}) and 0.2 ml of a col VB lysate, incubated for 20 min at 30 °C and titered on LB plates, seeded with 0.1 ml of the col VB lysate. The frequency of appearance of *tonB* clones was calculated from the ratio no. of clones on LB + col VB / no. clones on LB.

* Experiment performed at 42 °C: 10 *tonB* clones derived from the *Mu*c62 X lysogen were analysed. They all retained the immunity and S genes of the Mu prophage, showing that none of the deletions extended into the Mu prophage. This was done to check that none of the deletions was mediated by the *IS*1 present in the B gene of the *Mu*c62 X rather than by Mu itself.

Table 2 Translocation of the *asd* gene on to an *Flacpro* episome by *Mucts62 Aam1093* and *Mucts62 Bam1066* prophages inserted in *malA*

Donor	Frequency of <i>asd</i> ⁺ translocation
(<i>Mucts62 Aam1093</i>) <i>sup</i> ⁺ / <i>Flacpro</i>	4×10^{-8}
(<i>Mucts62 Aam1093</i>) <i>supF</i> ⁻ / <i>Flacpro</i>	3×10^{-8}
(<i>Mucts62 Bam1066</i>) <i>sup</i> ⁺ / <i>Flacpro</i>	2×10^{-8}
(<i>Mucts62 Bam1066</i>) <i>supF</i> ⁻ / <i>Flacpro</i>	5×10^{-8}

The strains were grown in LB broth at 30 °C to $\sim 2 \times 10^8$ ml⁻¹; 0.5 ml of both the donor *Flacpro/malA* :: (*Mucts62am*) *recA* *sup*⁺ or *supF*⁻ and the recipient *argG*, *metA*, *asd*, *thi*, *lac*, *recA*, *Rif*^R (*Muc*⁺), were mated for 2 h at 42 °C. *Asd*⁺ sexductants were selected by plating dilutions of the mating mixture on minimal medium supplemented with glucose, arginine, methionine and rifampicin. The frequency of *asd* transfer which is taken as the measure of Mu-mediated translocation, is given by the ratio no. of *asd* sexductants/input of *F'* cells.

in strains used as positive control, that is, the *supF*⁻ *trp* : : (*Mucts62 Aam*) and *supF*⁻ *trp* : : (*Mucts62 Bam*) in which the A and B mutations were suppressed, was $\sim 10^{-4}$. The same frequency was found with *sup*⁺ strains lysogenic for either *MuBam* or *MuX* mutants where the B gene was not expressed. However, in the *sup*⁺ strain lysogenic for *MuAam*, *tonB* clones appeared at a frequency of 10^{-8} , this being the same frequency as that found in the strain used as negative control (*trp* : : (*Mucts62*) (*Muc*⁺)) in which no thermal induction of Mu occurred.

This clearly shows that the A gene product is required for Mu-mediated deletion formation, while the B gene product is dispensable. Mu-mediated translocation occurs both after infection with Mu of a sensitive bacterium and in induced Mu lysogens. This can be readily detected in an *F'* strain, by transferring the episome to a recipient in which it is possible to select for plasmids which have acquired a given translocated marker. When the prophage is fully induced proximal and distal host markers are translocated at the same frequency⁷. We tested the ability of *A*⁻ and *B*⁻ induced Mu prophages to mediate translocation of proximal markers. We isolated *sup*⁺/*F'* *pro lac* strains, carrying either a *Mucts62 Aam* or a *Mucts62 Bam* prophage in the *malA* gene, and their *sup*⁻ derivative which were used as positive controls. These strains were mated at 42 °C with a recipient suitable for selection of transferred *F'* having eventually acquired and *asd* gene (which is located near *malA* (ref. 11)). The results in Table 2 show that translocation of the *asd*⁺ allele occurs at a frequency of 3.5×10^{-8} in the *sup*⁻ lysogens, while in the *sup*⁺ lysogens this frequency is $\sim 4 \times 10^{-8}$, thus ~ 100 times lower. Translocation by an induced *Mucts62 X* prophage was also tested (data not

shown) and was found to be negative. From these results we conclude that both the A and B gene products are required for Mu-mediated translocation.

As an alternative approach, prophages affected in their ability to mediate translocation, were isolated after treatment of a *Mucts62* lysogen with nitrosoguanidine. The strain used contains an *F' lac* episome, and a *Mucts62* prophage in the *trp* operon. After mutagenesis we screened for clones which after induction neither produced viable phage nor transferred episomes with translocated markers. We shall ignore mutants which were found to directly affect the transfer of the *F'* episome (4% of the total number of clones screened). Ten mutants (1.3% of the clones tested) in which the deficiency was found to be in the Mu prophage were analysed for their ability to mediate deletion of the *tonB* chromosomal marker and to translocate markers proximal to the prophage, on to the *F lac* episome. They were also tested for their capacity to complement *A*⁻, *B*⁻ and *L*⁻ mutants of Mu. All mutants fall into two classes (see Table 3): some (2/10) complement *A*⁻ and *L*⁻, but not *B*⁻ mutants of Mu, could mediate formation of *tonB* deletions and could not mediate translocation; the others (8/10) complemented the *L*⁻ mutant; some complemented *B*⁻ mutants but none complemented *A*⁻ mutants, nor could any mediate either deletion formation or translocation of chromosomal markers. This confirmed the findings that the B gene product is not required for deletion formation while the A gene product is necessary, and that both A and B products are essential for Mu to mediate translocation. Mu-mediated deletions and insertions are events which can occur in an induced Mu lysogen at the site where the prophage is originally located and therefore do not necessarily involve transposition of Mu DNA. On the other hand, Mu-mediated translocation always involves transposition of the Mu genome. On the basis of our previous work⁸, we conclude that two Mu copies are always involved in Mu-mediated illegitimate recombination. As long as the Mu prophage is integrated into the host chromosome which is the case in the induced lysogen since no extensive excision of the Mu genome occurs after induction¹³, the two copies of Mu can be generated through the replication of the host genome. However, as soon as Mu leaves the host chromosome, most probably as a heterogeneous circle containing host DNA covalently linked to one Mu copy¹⁴⁻¹⁶ it must be able to replicate by itself to generate two copies. One would thus expect Mu replication function(s) to be dispensable for those chromosomal rearrangements which can occur at the site of the prophage, that is, deletions and insertions, but these

Table 3 Properties of the prophage mutants selected for their inability to transpose host markers

Mutant isolated	Frequency of translocation on <i>Flac</i> of		Frequency of <i>tonB</i> deletion	Complementation of mu mutants in gene		
	<i>pyrF</i> ⁺	<i>thyA</i> ⁺		<i>A</i>	<i>B</i>	<i>L</i>
1	2×10^{-7}	3.4×10^{-8}	5.3×10^{-6}	—	+	+
2	1.6×10^{-6}	9×10^{-8}	5×10^{-6}	+	—	+
3	1.5×10^{-7}	6×10^{-7}	23×10^{-6}	—	+	+
4	1.6×10^{-7}	10^{-6}	1.1×10^{-4}	+	—	+
5	8×10^{-8}	1.6×10^{-7}	1.7×10^{-6}	—	+	+
6	2.4×10^{-7}	1.6×10^{-8}	4.7×10^{-6}	—	—	+
7	4.7×10^{-7}	2.9×10^{-6}	2.2×10^{-6}	—	—	+
8	8×10^{-8}	2.5×10^{-6}	6.5×10^{-6}	—	—	+
9	2.4×10^{-7}	4×10^{-6}	5.2×10^{-6}	—	—	+
10	1.2×10^{-7}	2×10^{-8}	4.5×10^{-6}	—	+	+
<i>Mucts62</i>	2.3×10^{-8}	8×10^{-8}	1.5×10^{-4}	+	+	+

MuA⁻ is *Mucts62 Aam1093* (ref. 19). *MuB*⁻ is *mucts62 Bam1066* (ref. 19), *MuL*⁻ is *Mucts62 Lts5017* (ref. 20). The strain from which the mutants were derived contained a *Mucts62* prophage in the *trp* operon and an *Flac* episome. The method for measuring the *tonB* deletion frequency is described in Table 1. The frequency of translocation of the *pyrF*⁺ or *thyA*⁺ alleles on to the *Flac* were determined as described in Table 2 using a *pyrF*⁻, *thyA*⁻, (*Muc*⁺), *Spc*^R *recA*⁻ recipient. Complementation tests were performed by introducing *F' lac Mucts62am* episome in *F*⁻ *recA*⁻ derivative of the mutated lysogens and measuring phage production on *sup*⁺ and *sup*⁻ indicator bacteria, after induction of the dilysogens.

functions should be essential for translocations which always involve transposition of Mu DNA and duplication of the phage genome outside of the host chromosome. This is the case for the B gene product, and therefore we propose that the B gene product is involved in Mu replication. Mu integration function(s) are, on the contrary, expected to be required for all Mu-mediated chromosomal rearrangements. Thus, as only A^- mutants of Mu are unable to mediate deletion formation, integration of extrachromosomal DNA and translocation, we propose that the A gene product is an integration function. The same conclusion has been reached independently by O'Day *et al.*²¹: they found that only MuA^- mutants are completely defective for integration.

Part of this work was completed while M.F. was a post doctoral fellow at the Departamento de Investigaciones Biomédicas, UNAM, Mexico; O.H. was a EURATOM fellow; A.T. is Chargé de Recherches from the Belgian FNRS.

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Received 3 October; accepted 9 December 1977.

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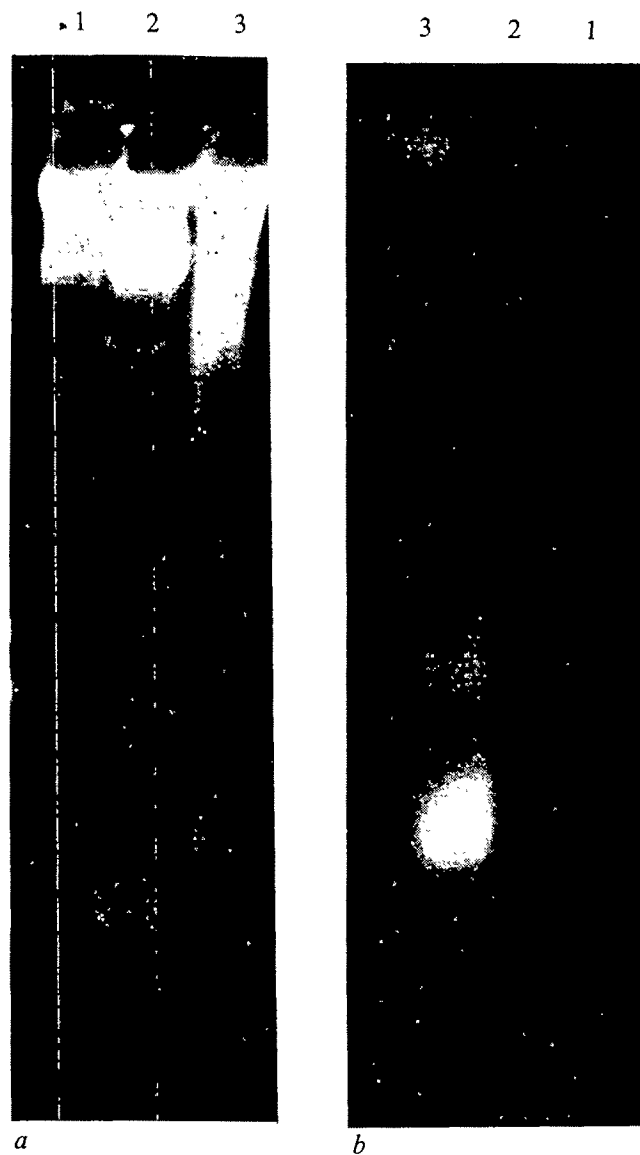
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Immunoglobulin light-chain structural gene sequences cloned in a bacterial plasmid

IMMUNOGLOBULIN light-chain mRNA molecules and their cDNA transcripts have served as molecular probes in hybridisation experiments designed to quantify the number of immunoglobulin genes¹⁻¹³ and to characterise the arrangement of these genes in the mammalian genome^{14,15}. In other studies these mRNAs and cDNAs have been used as substrates for nucleotide sequence analyses to define the structural gene encoding

immunoglobulin light chains^{16,17}. Unfortunately, two central technical problems have developed during these studies. First, the immunoglobulin mRNA cannot be obtained in amounts sufficient for many experiments. For example, determination of the complete nucleotide sequence of this RNA remains difficult without more material. Second, immunoglobulin mRNA cannot be obtained as a homogeneous RNA completely free of other RNA species. These impurities have probably interfered with several hybridisation studies. In order to obtain a substrate for nucleotide sequence analysis as well as to provide a pure hybridisation probe we have cloned the DNA (cDNA) complementary to the immunoglobulin light-chain (kappa) mRNA from the mouse myeloma MOPC-149. The

Fig. 1 DNAs from plasmids (1) pCRI, (2) pBG2 (ref. 20), and (3) pCRI- κ 40 were digested with S1 nuclease in 40% formamide and fractionated on a 2% agarose gel run from top to bottom. The exact conditions for digestion of the DNA are as described by Hofstetter *et al.*²⁵. *a*, Bands identified by ethidium bromide staining. *b*, An autoradiogram obtained after the DNA from the lanes shown in (*a*) was denatured and transferred to a nitrocellulose filter, and hybridised to ³²P-MOPC-149 cDNA as described by Southern²⁷. The size of the pCRI- κ 40 insert fragment was determined to be 700 base pairs by calibration with *Hae*III fragments of ColEI DNA (marker fragments not shown). The pBG2 insert fragment (*a*, lane 2) is 576±50 base pairs (ref. 15).



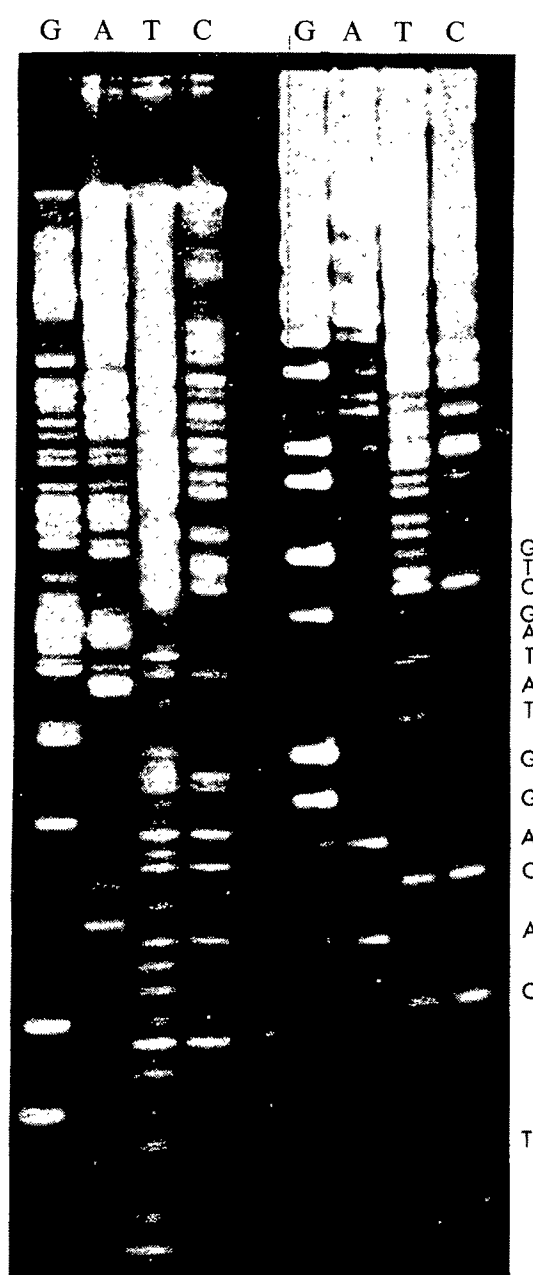


Fig. 2 Radioautograph for determination of the nucleotide sequence starting from the *Hae*III site in *pCRI-κ40* reaching towards the *Pst*I site. The 850-base pairs *Hae*III fragment found in digests of *pCRI-κ40* was isolated from an acrylamide gel, kinased, and cleaved with *Pst*I (see Fig. 3 for location of restriction sites). The 325-base pair fragment generated in this way was then subjected to the reactions described by Maxam and Gilbert³⁰. G, A, T, C across the top of the radioautograph indicate the specificity of the reaction. Two sets of reactions were electrophoresed for 19 h (a) and 8 h (b). The letters on the right side of the figure indicate the nucleotide sequence suggested by the band pattern. A few of the deduced residues are indicated as examples. These nucleotides correspond to residues 3–17 from the *Hae*III cleavage site. The complete sequence deduced from these autoradiograms is shown in Fig. 3.

plasmid *pCRI-κ40* is shown here to contain sequences corresponding to 700 bases of the immunoglobulin mRNA.

The cDNAs derived from several polyadenylated mRNAs have been converted into double-stranded DNA and joined to plasmid vectors using the poly dA-T tailing procedure¹⁸ or the synthetic oligonucleotide linker method¹⁹. The insertion of rabbit β-haemoglobin²⁰ and rat insulin¹⁹ mRNA sequences into plasmids has been confirmed by nucleotide sequence

analysis of the cloned DNA segment. Other insert carrying plasmids have been shown to hybridise to MOPC-173 immunoglobulin light-chain cDNA²¹, mouse α and β haemoglobin cDNA²², and ovalbumin cDNA²³, suggesting that the sequences of these mRNAs have also been cloned in plasmids. We used a slight modification of the poly dA-T tailing procedure, first used for cloning the rabbit β haemoglobin cDNA^{18,20} sequences, to clone immunoglobulin cDNA and have unambiguously confirmed its presence in the clone by determining a portion of its nucleotide sequence.

We began with the total poly A-containing mRNA found on the polysomes of the mouse myeloma tumour MOPC-149 rather than pure immunoglobulin mRNA. (The purest fractions of 14S mRNA were reserved for the screening procedures used to identify clones containing immunoglobulin sequences.) Immunoglobulin light-chain mRNA is only 10–30% of this RNA population. The cDNA obtained by reverse transcription of this population of mRNAs was found to be very heterogeneous in size, 50–2,000 bases in length (J.G.S., unpublished). Nevertheless, we used this heterogeneous cDNA as a substrate for *Escherichia coli* DNA polymerase I to generate double-stranded DNA hairpin structures. These hairpin structures were converted into double-stranded DNA by digestion with S1 nuclease. Then, poly dA stretches were attached to the double-stranded DNA, and poly dT stretches were added to linear plasmid *pCRI* DNA (which had been cleaved with *Eco*RI) by the action of terminal nucleotidyl transferase (kindly provided by R. L. Ratliff) using the procedure described by Wu *et al.*²⁴. Finally, the poly dA 'tails' on the double-stranded DNA were annealed to the poly dT 'tails' of the plasmid DNA to form circular hybrid molecules.

In this way a large number of hybrid molecules were formed. To amplify and purify a hybrid molecule containing immunoglobulin gene sequences we used these hybrids to transform *E. coli*. The NIH Advisory Committee on Recombinant DNA has decided that this type of experiment requires an EK2 host-vector system and a P3 level of physical containment. We complied with these requirements. We therefore used the crippled EK2 host, *E. coli* strain κ1776, with the approved bacterial plasmid *pCRI*. When 0.8 μg of hybrid molecules were used to transform *E. coli* strain κ1776 we obtained 112 independent transformed clones (that is, cells which had received the kanamycin resistant phenotype conferred by *pCRI*).

As expected, not all of these 112 clones contained immunoglobulin sequences. These 112 clones were grown on nitrocellulose filters, and hybridised to highly purified ¹²⁵I-labelled

Table 1 Extent of hybridisation of MOPC-149 cDNA to various plasmid DNAs

Unlabelled sequences	MOPC-149 ³ H-cDNA S1-nuclease resistant c.p.m. (%)
<i>pCRI-κ40</i>	135 (66)
<i>pCRI-λ52</i>	21 (10)
<i>pCRI</i>	32 (16)
MOPC-149 mRNA	209 (100)

Full-length, purified ³H-MOPC-149 cDNA was hybridised to an excess of unlabelled plasmid DNA, and the amount of hybrid formed was measured as the amount of S1-nuclease resistant material formed during hybridisation²⁵. The plasmid *pCRI-λ52* was constructed in the same fashion as plasmid *pCRI-κ40*, except that total poly A-containing mRNA from the myeloma tumour RPC-20 was used to make the initial cDNA transcripts. 200 c.p.m. of MOPC-149 cDNA was added to 0.5 μg sonicated plasmid DNA or 20 ng of MOPC-149 mRNA, in 0.6 M NaCl, 50 mM Tris pH 7.5, in a final reaction volume of 50 μl. The samples were boiled, placed at 70 °C and allowed to hybridise for 2 h. Finally, the samples were diluted into 1 ml of S1-nuclease buffer (0.04 M NaCl, 0.03 M sodium acetate pH 4.5, 0.12 M ZnSO₄) divided into two equal portions and one portion was digested with 5 μl S1-nuclease for 1 h at 45 °C. The number of trichloroacetic acid-precipitable counts remaining after S1 digestion was taken as a measure of the amount of hybrid formed.

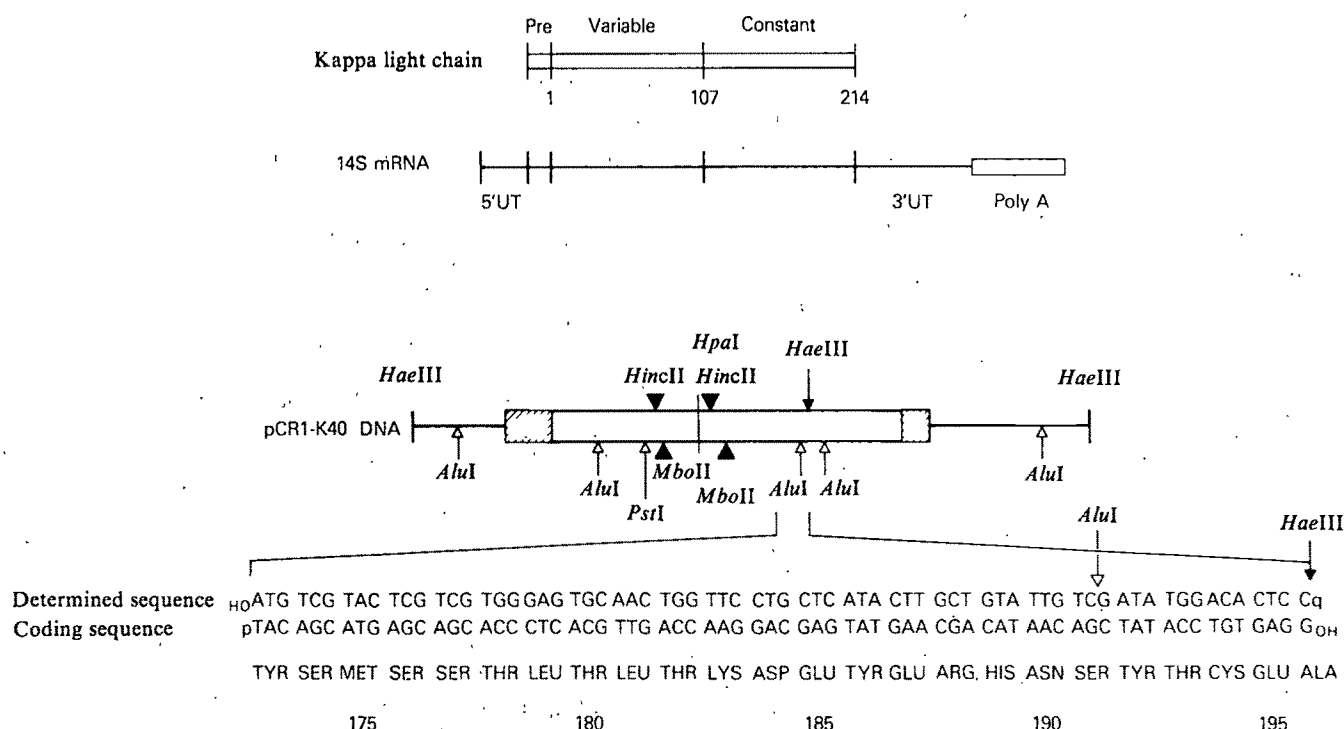


Fig. 3 The arrangement of the immunoglobulin light-chain gene sequences inserted into pCR1- κ 40 and the location of restriction enzyme sites in the inserted sequence. Kappa light chain represents a single polypeptide (amino terminus left; carboxyl terminus right) formed during *in vitro* translation of 14S immunoglobulin mRNA. The polypeptide has been divided by vertical lines into three regions, a 20-amino acid putative precursor sequence, a 107-amino acid variable region sequence and a 107-amino acid constant region sequence. The numbers below the vertical lines indicate the amino acids at the ends of the variable and constant regions. 14S immunoglobulin light-chain mRNA, drawn with its 5' end at the left, is roughly 1,200 bases long (including about 200 bases of poly A at the 3' end). The regions directly below the variable and constant regions of the light chain represent the two 321-base sequences required to encode these portions of the polypeptide. 5' UT and 3' UT are the parts of the mRNA which are not translated into protein, and are found near the 5' and 3' ends of the mRNA. The pCR1- κ 40 DNA segment shown includes the insert sequence, as well as the plasmid sequences (represented by a straight line) and the poly dA-T linkers (cross-hatching) that join the insert to the plasmid. The determined nucleotide sequence (see Fig. 2) was found to be from the anti-sense strand. The coding sequence, deduced from the anti-sense sequence, was found to correspond to amino acids 173-196 of the constant region (bottom row). Most of the restriction enzyme sites, (*Pst*I, one *HincII*/*Hpa*I, one *Mbo*II, and one *Alu*I site) were determined by digesting the 325-base pair fragment or the 525-base pair fragment, obtained by *Pst*I cleavage of the kinase-treated 850-base pair *Hae*III fragment, with each enzyme. Similar multiple enzyme digests using kinase-labelled fragments were used to identify the other indicated cleavage sites. *Mbo*II, and *Alu*I each cleave the insert sequence in at least one more location, but these locations have not been defined. We have noted that the deduced sequence contains a *HincII* recognition sequence (at the sequence encoding amino acid 178/179), however while some evidence for partial cleavage at this site has been found, the enzyme prefers the other two sites at least threefold.

14S mRNA obtained from MOPC-149 myeloma tumours using the procedure of Grunstein and Hogness³¹. Sixteen of the 112 clones hybridised to purified mRNA (data not shown). The DNAs from these 16 clones were then screened for their ability to protect highly purified MOPC-149 cDNA from S1-nuclease digestion. For example, the DNA from pCR1- κ 40 was able to protect 66% of a MOPC-149 cDNA from S1-nuclease digestion (Table 1). pCR1- κ 40 DNA protected more MOPC-149 cDNA than the DNAs from any of the other 15 clones, so this clone was chosen for further analysis.

One problem with the poly dA-T joiner method is that insertion of a sequence at the restriction endonuclease *Eco*R1 cleavage site within the plasmid DNA destroys the *Eco*R1 site. Thus the insert sequence cannot be cleanly excised from the plasmid DNA by the action of a single restriction enzyme. To overcome this problem Weissman and co-workers²⁵ have developed a technique which cuts the poly dA-T joiner sequences and excises the insert sequence as a single fragment. As poly dA-T has a lower melting temperature than any other sequence, these sequences can be preferentially denatured in 40% formamide. These sequences are then susceptible to S1-nuclease digestion. The fragment excised in this way can be separated from the rest of the plasmid DNA by gel electrophoresis. The results of such an experiment performed on pCR1- κ 40 DNA are shown in Fig. 1a. Two fragments are excised from

pCR1- κ 40 DNA, but not from pCR1 DNA. Both of these fragments specifically hybridise to MOPC-149 cDNA (Fig. 1b). We suggest that the fainter, slower band is a partial digestion product, reflecting the presence of an easily denatured sequence in the plasmid pCR1. We believe that the faster-moving fragment, present in high yield, contains the insert sequence free of any plasmid sequences. The size of the inserted sequence seems to be 700 ± 50 base pairs. Consistent with this result was the finding that the R loop²⁶ formed between pCR1- κ 40 DNA and MOPC-149 mRNA was about 700 base pairs long (J.G.S., M. Sullivan, and B. Peterlin, unpublished).

The sequence inserted into pCR1- κ 40 is only 700 ± 50 bases long whereas immunoglobulin mRNA (not including the poly A) is 950 bases long, indicating that about 200 bases of light-chain mRNA sequences must be missing from the cloned sequence. Which portions of the mRNA are not included in the inserted sequence in pCR1- κ 40? To answer this question, and in order unambiguously to identify the cloned segment, we identified the restriction endonuclease cleavage sites in the inserted sequence, determined the nucleotide sequences around these cleavage sites, and then correlated the nucleotide sequences with the amino acid sequence which they encode.

We have found six restriction endonucleases that cleave the inserted sequence in pCR1- κ 40. pCR1 and pCR1- κ 40 DNA were digested with many restriction enzymes and electro-

phoresed on polyacrylamide or agarose gels; enzymes with cleavage sites in the insert sequence showed at least one more band in the pCR1- κ 40 digests than in the pCR1 digest. These results were confirmed by blotting the fragments onto nitrocellulose paper and hybridising to ^{32}P -labelled MOPC-149 cDNA as described by Southern²⁷. If an enzyme cleaved the insert sequence then label would hybridise to two bands rather than just a single band. In this way the restriction enzymes *Mbo*II, *Alu*I, *Hae*III, *Hpa*I, *Hinc*II, and *Pst*I were each shown to cleave the inserted sequence at least once, while the enzymes *Bam*HI, *Hind*III, *Hha*I, *Sst*I, *Hinf*I, *Hae*II, *Hpa*II, *Kpn*I and *Bgl*II do not seem to cleave the inserted sequence.

The nucleotide sequence of a small portion of the inserted sequence in pCR1- κ 40 was determined in order to demonstrate conclusively that the inserted sequence could encode for a portion of light-chain amino acid sequence. An 850-base pair fragment from a *Hae*III digest of pCR1- κ 40 DNA was isolated, labelled at its 5' ends with ^{32}P , cleaved into two pieces (a 525-base pair fragment and a 325-base pair fragment) with *Pst*I (see Fig. 3), and finally the 325-base pair fragment was subjected to the standard reactions for sequencing DNA fragments of Maxam and Gilbert³⁰ (Fig. 2). The nucleotide sequence determined from this procedure is shown in Fig. 3. This nucleotide sequence when read in the correct frame correlates perfectly with a 23-amino acid sequence from the immunoglobulin kappa-type constant region, and with the RNA sequence predicted by Milstein *et al.*²⁸. Thus, we conclude that an immunoglobulin sequence has been inserted into pCR1- κ 40. Using this nucleotide sequence to orientate the map we were able to determine the arrangement of immunoglobulin sequences in pCR1- κ 40 (Fig. 3). Further, the plasmid pCR1- κ 40 contains about 700 base pairs of the immunoglobulin gene sequence, spanning a portion of the 3' untranslated region, the entire constant region and over 150 base pairs of MOPC-149 variable region gene sequence.

This hybrid molecule will be of great value for nucleotide sequence analysis, identification and cloning of genomic DNA segments containing actual immunoglobulin genes and the unambiguous determination of their arrangement in chromosomal DNA. Indeed, we have already used pCR1- κ 40 to obtain two interesting chromosomal DNA fragments containing immunoglobulin variable region sequences (J.G.S., M.H.E., D. Tiemeier, S. Tilghman, F. Polsky, A. Leder, P.L., unpublished).

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Corrigendum

In the letter 'Detection of casein messenger RNA in hormone-dependent mammary cancer by molecular hybridisation' by J. M. Rosen & S. H. Socher, *Nature* **269**, 83-86, the legend to Fig. 4 should start 'Measurement of casein mRNA in hormone-independent mouse mammary tumours. . . .' (not hormone-dependent). Similarly, line 2 on page 86 should read '. . . in the hormone-independent mouse mammary carcinomas . . .'.

Errata

In the letter 'Birefringence signals and calcium transients in skeletal muscle' by G. Suarez-Kurtz & I. Parker, *Nature* **270**, page 748, the horizontal calibration in Fig. 3a should read 50 ms, not 10 ms.

In the letter 'Circular structures of large scale and great age on the Earth's surface' by J. M. Saul, *Nature* **271**, 345-349, Figs 1 and 2 were reproduced to the wrong scale so that the transposition marks do not correspond. The scale reduction of 1:1,000,000 in Fig. 1 legend is also incorrect. The correct versions of these figures can be obtained in reprints of the letter.

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matters arising

Biostratigraphy of Seymour Island, Antarctica

THE paper by Hall on Cretaceous and Tertiary dinoflagellates from Seymour Island, Antarctica¹ contains misleading information. In particular, the stratigraphic columns in Fig. 1 do not represent the known field relations. The overlap of sections S-13 and S-3 seems most unlikely on geological grounds; the sections are less than 3 km apart, the sediments have different provenances, and represent different facies (S-13 is delta plain, and the lower part of S-3 is prodelta or delta slope, and palaeo-current indicators suggest southeastward flow). The base of the measured section S-3 is not the base of that part of the sequence. Section S-11 occurs below the unconformity above which S-13 is located; the Palaeocene age may well be correct, but there is no overlap in time with S-13. The age of the beds at Cape Wiman (S-16) is open to debate, but the correlation indicated in Fig. 1 is an oversimplification of the possible interpretations.

The ammonite-dated strata form part of a homoclinal sequence dipping to the south-east and occupying the whole of the southern half of Seymour Island. Howarth² figured several ammonites from near the base of the section, and one ammonite from near where the unidentified ammonite was found that contains 'early Senonian' dinoflagellates in the matrix. Howarth assigned an Early to Middle Campanian, or possible late Campanian age, to the ammonites he described. The strata from which the 'unidentified' ammonite comes are unconsolidated, so that fossils are found, with few exceptions as loose material on dip slope surfaces or at the base of short sections. The ammonite was collected on a dip slope. The southern half of Seymour Island, unlike the northern part north of Cross Valley, lacks glacial debris, and therefore the ammonite cannot be considered a glacial erratic. The 'unidentified ammonite' belongs to the genus *Maorites*, is identified specifically as *M. densicostatus* (Kilian and Reboul), and others of this species are figured by Howarth². Hall's postulate of an 'early Senonian' age seems to be in conflict with the ammonite data. Until proven otherwise, the most likely explanation of this apparent conflict is that the dinoflagellates had been

reworked from older strata or, as stated by Sarjeant³, that the exact ranges of these dinoflagellate species are uncertain.

Finally we would note that Simpson⁴ regards the so-called Miocene beds as Late Eocene, though possibly either Middle Eocene or Early Oligocene. Furthermore, Von Ihering³ recognised the Eocene affinities of the molluscs.

Hall's data are very valuable, but some of the statements and conclusions are misleading for those not familiar with the local geology. Papers on the stratigraphy and palaeontology of the Tertiary rocks on Seymour Island have been presented at the Third Symposium on Antarctic Geology and Geophysics at Madison, Wisconsin, in August, 1977.

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HALL REPLIES—In 1975 I examined sediment samples from four measured sections on Seymour Island, Antarctic Peninsula in a pilot study of dinoflagellates, spores, and pollen. A series of palynologic assemblages from two sections, S-3 and S-11, contained numerous diagnostic dinoflagellates; the sections were provisionally assigned a late Eocene-early Oligocene age and a Palaeocene age, respectively. However, sections S-13 and S-16, the correlations of which Elliot and Zinsmeister are critical, are represented collectively only by three samples. Concerning age dating, I reported that "neither of the samples from S-13 provides conclusive information". I also suggested a Palaeocene or late Cretaceous age for S-16 based on one dinoflagellate assemblage. Not only did I report the unsureness of the correlations of S-13 and S-16, but question marks were drafted adjacent these sections in Fig. 1 of ref. 1, further stressing the uncertainty of the correlation of these two sparsely sampled sections. The correlations shown in the figure that are misleading to Elliot and Zinsmeister are clarified on reading the text of the article.

The dinoflagellate species *Cyclonephelium distinctum* and *Deflandrea cretacea* have greater ranges than I acknowledged in the article. Thus my

age assignment of 'early Senonian' to the ammonite matrix (unidentified when I submitted the manuscript but subsequently referred to *Maorites densicostatus*) is indeed unwarranted.

Elliot and Zinsmeister cite Simpson's⁴ review of fossil penguin material and Von Ihering's³ 1927 molluscan studies from Seymour Island as previous interpretations of an Eocene age for these beds, an implied criticism that my corresponding conclusion based on dinoflagellates is less than original. I give Simpson full credit for his conclusions in my article: "The dinoflagellates evidence supports Simpson's conclusion that the fossil penguin materials from Seymour Island, presumably from the upper part of section S-3 or its equivalent, are no older than late Eocene and no younger than early Oligocene". Von Ihering's fortuitous interpretation in 1927 of an Eocene age for the Seymour Island Series was disputed by Antarctic scholars for over 40 yr; the beds were regarded as Miocene in age⁴ until Simpson's vertebrate work and the supporting evidence of the dinoflagellates.

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Asymmetrical displacement currents

KOSTYUK *et al.*¹ have recorded an asymmetrical displacement current in snail neurones (*Helix pomatia*) which, because of a similar voltage dependence, they have associated with the calcium conductance change caused by membrane depolarization. It was noted that the characteristics of the 'calcium gating currents' in snail¹ and *Aplysia*² neurones were different, and it was suggested that at least part of the difference was due to a technical problem with series resistance. However, we think that there are indeed actual differences in the displacement currents recorded in the two neurones.

First, in voltage-clamp studies in snail neurones, it has not been possible to demonstrate separate channels with

different kinetics or voltage dependence for sodium and calcium ions³⁻⁵. Consequently the displacement current recorded in snail neurones¹, although sharing the voltage dependence of calcium ionic current, may be associated with gating of sodium channels, gating of calcium channels, or gating of common channels through which both sodium and calcium ions can pass. In contrast, separate, distinguishable channels for sodium and calcium ions have been demonstrated clearly in *Aplysia* neurones because of differences in kinetics and voltage dependence^{2,6}. Thus a displacement current with properties (particularly voltage dependence) similar to those of the calcium conductance change, but distinct from those of the sodium conductance change can reasonably be called a calcium gating current. If the displacement current recorded in snail neurones¹ is not gating channels selective for calcium ions, as seems possible, it could well be different from the calcium gating current in *Aplysia* neurones.

Second, we believe that the slow rising phase of the calcium gating current recorded in *Aplysia* at 6°C is real and not due to slow establishment of a new clamp potential, as suggested by Kostyuk *et al.*¹, for the following reasons. (1) As we have stated², new clamp potentials were reached within 100 μ s. (2) The measured series resistance was always less than 0.5% of the membrane resistance. We have calculated that the time constant of the change in membrane potential across the surface membrane would be less than 100 μ s. (3) The rising phase of the calcium gating current is preceded by an 'immediate on, exponential off' asymmetrical displacement current which we believe is sodium gating current for the reasons given². If the slow rising phase of the following calcium gating current were due to slow establishment of the clamp potential, the time course and voltage dependence of the earlier displacement current would be difficult to explain. (4) The time to peak of the calcium gating current has a high Q_{10} of about 3. This temperature sensitivity can hardly be due to slow establishment of new potential levels across the surface membrane. (5) The time to peak of the calcium gating current can be varied by changing the holding potential while keeping the clamp step amplitude constant, again difficult to explain in terms of slow clamping.

We must conclude therefore that the slow rise of calcium gating current we have recorded in *Aplysia* neurones² is real and cannot be explained as suggested by Kostyuk *et al.*¹ If the displacement current recorded by

Kostyuk *et al.*¹ is indeed a true calcium gating current, there may be real differences in calcium gating currents in the surface membranes of the two nerve cells.

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KOSTYUK *et al.* REPLY—Adams and Gage have suggested several additional arguments in favour of the existence of a rising phase in the development of Ca gating current recorded in the neurone of *Aplysia*. But they do not consider the problem of membrane potential settling time in the two microelectrode voltage clamp experiments. They say that the "control of membrane potential . . . was achieved in less than 100 μ sec". But the rapid changes of potential measured at the microelectrode input can strongly differ from the real (and much slower) changes in the membrane potential. This difficulty in microelectrode voltage clamp experiments has been mentioned by Geduldig and Gruener² in their investigation of *Aplysia* neurones. It is due to the existence of a capacitance between closely situated microelectrodes used to measure potential and inject feedback current. Detailed analysis of the corresponding circuit is quite complicated (some useful information can be found in ref. 3). The settling time of a new clamp potential can be estimated from the termination of current capacitive transient only. The membrane current curves demonstrated in Fig. 1 by Adams and Gage¹ (although presented on a contracted time scale) suggest that the capacitive transient in these experiments lasted for several ms. The time-to-peak of the asymmetrical current presented in Fig. 3 is in qualitative agreement with that length.

These arguments are not intended to question the relationship of the recorded asymmetrical current¹ to the movement of gating charges. But it seems likely that the rate of decay of the 'early' component and the delayed rising phase of the 'late' component reflect the conditions of the experiment rather than the real kinetics of gating currents. Other effects suggested as

arguments in favour of the 'natural' origin of the rising phase can also be explained in another way. Thus the cooling of the preparation results in an increase in membrane resistance. It is equivalent to an increase in the internal resistance of the current source which charges the membrane capacitance.

The discovery of a true rising phase in the gating current would be very important for the description of gating charge movement⁴. But the experimental problems of sufficiently fast membrane potential settling are difficult to solve and must be treated cautiously⁵. As to the separation of specific Na and Ca channels in molluscan neurones, this was achieved recently with the help of the intracellular dialysis technique⁶.

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Multispecific antibodies

VARIOUS points were raised by Secher¹ in a comment on our recent letter on "Evidence for multispecificity of antibody molecules²."

One question concerned the biological significance of cross reactions having binding constants of about 10^3 l mol⁻¹. We showed that these cross reactions will fix complement and lyse red cells²; one can conclude that if they can induce cytolysis, they are likely to be of biological significance. On the other hand, the concentration of antibody (and target antigen) has an important role. If one assumes a titre of 150 μ g ml⁻¹ of IgG (that is, 10^{-6} M) and, for simplicity, a similar one for the antigen (although if it is on a cell membrane one really cannot make an estimate), then, for a binding constant of 10^3 l mol⁻¹, about 10% of the antibody will be in the bound form. This is a conservative estimate since it has been shown^{3,4} that the 'functional' affinity is larger by a factor of 10^3 to 10^4 than the association constant for the binding of a monovalent hapten. The binding interaction would be even more favoured if the antigen were concentrated in a particular area, for example, bacterial antigens in a local infection, or, as in the case of putative autoimmune diseases, antigenic structures on tissues and organs.

Secher calls our analogy between antibodies and enzymes "not very appropriate" because "enzymes are homogenous and so multispecificity in substrate binding could not enhance their specificity . . .". Perhaps we were unclear in our paper. We were not trying to draw a functional analogy but were trying to indicate that all protein-ligand interactions rely on the same play of short range forces and geometry. Hence, if enzymes are multispecific, it should not be surprising to find that antibodies are also multispecific.

We should like to comment also on Secher's suggestion that the best antibodies (with binding constants of 10^9 – 10^7 mol⁻¹) may be highly specific (that is, not multispecific). From our point of view, this is not a logical conclusion. Whether a binding constant for a ligand is 10^9 mol⁻¹ or 10^6 mol⁻¹, the character of the binding site of the antibody is no different. It still should be able to accommodate other ligands and interact with them. Presumably the binding constant of a cross reaction could be even higher than that of the reaction with the immunogen.

We should like to thank Secher for reminding us of the early suggestions of Talmage⁵. We are embarrassed by our failure to cite his paper.

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SECHER REPLIES—Enzymes (and antibodies) can bind ligands (antigens) other than the 'primary ligand'. Ligands with very similar chemical structure may bind almost as tightly as the main ligand and one might expect to find a series of compounds of decreasing association constant and decreasing chemical similarity to the primary ligand. The concept of multispecificity (as used by Inman for example) is different. It implies that two or more antigens of unrelated structure can bind specifically to a single antibody molecule. This could take place at distinct sites, overlapping sites, or the same site, but presumably will use different contacts between the antibody and the antigen.

The former phenomenon is well known for both enzymes and antibodies. However there are only a few claims of examples of multispecificity

(in both systems), and even these are controversial.

With reference to the specific points that Cameron and Erlanger raise above. Binding of antigen is only one aspect of the biological significance of a cross-reaction. The ability to trigger lymphocytes into division and antibody production must also be considered, and also the extent to which such cross-reactions are representative of the total antibody population or are rare exceptions.

I agree that if "enzymes are multispecific then it should not be surprising to find that antibodies are also multispecific". To what extent these generalities have been demonstrated is still not clear (see above for definition of multispecificity).

The final sentence of my original article was intended to be taken as pure speculation and not as a logical conclusion.

Cross-reactions with binding (association) constant higher than that of the immunogen do indeed exist and have been named 'heteroclitic'.

(An error in my original article has been corrected²).

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Marsupial trophoblast and mammalian evolution

We present here a view of marsupial-eutherian origins and evolutionary trends, which constitutes an alternative to the view of Lillegraven^{1,2} recently discussed by Cox in a *Nature News and Views* article³.

The trophoblast constitutes the outer foetal boundary of the eutherian chorioallantoic placenta where it functions as the major foetal component of the placental barrier as well as a site of endocrine activity. The trophoblast is also a foetal component of the eutherian yolk sac placenta. In most marsupials, maternal-foetal transfers occur solely through a yolk sac placenta where trophoblast associates with both vascular and avascular regions. An important exception occurs in the marsupial bandicoots where both yolk sac and chorioallantoic placentae develop; trophoblast differs in form regionally within the boundaries of each type of placenta⁴⁻⁶.

The trophoblastic layer persists until term in the chorioallantoic placenta of all mammals except bandicoots, in which the layer disappears shortly before term⁴⁻⁶. Lillegraven, however, states (page 713, ref. 2) that "tropho-

blast is strictly found only in placental mammals," thus misquoting his authorities, Davies and Hesseldahl, who say that trophoblast is a peculiar mammalian structure. Lillegraven (page 720, ref. 2) states further that "The 'invention' of trophoblastic tissues by primaeval eutherians was probably the single most important evolutionary event in the history of the infraclass". This has led to the mistaken conclusion that it is the evolution of trophoblast which enables only Eutheria to obviate immunological crisis during pregnancy^{2,3}.

Lillegraven says (page 101, ref. 1) that "... true implantation by erosion of the maternal epithelium never occurs in marsupials". It has long been known that in the bandicoot chorioallantoic placenta trophoblast disappears as a layer late in gestation⁴⁻⁶, probably through fusion with the uterine luminal epithelium⁶. Comparable fusion may also occur during early implantation of certain Eutheria⁶. Similar, less extensive invasion of uterine epithelium by trophoblastic cells occurs at the yolk sac placenta in several marsupials (such as in *Dasyurus viverrinus* and *Sminthopsis crassicaudata*), the uterine epithelial layer becoming modified at the placental site by interaction of foetal and maternal tissues during implantation.

Thus, evidence concerning trophoblast and uterine erosion tends to unite rather than separate evolutionary aspects of eutherian and marsupial placentation. Although it is not known whether marsupial trophoblast contains immunological or endocrine properties comparable with those proposed for eutherian trophoblast⁷, attention is being focused on this important problem⁸. Because trophoblast is certainly not a eutherian innovation, the argument for the "competitive inferiority" of marsupials is considerably weakened, as Kirsch⁹, on other grounds, has also concluded.

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reviews

Bridging the two cultures

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A Sense of the Future. By J. Bronowski. Pp. 286. (Massachusetts Institute of Technology Press: Boston, Massachusetts and London, 1977.) \$12.50; £7.25.

JACOB BRONOWSKI was one of those rare people who deserve the title of "Renaissance Man". Academics are suspicious of Renaissance Men. A mathematician who can write perceptively about Blake, compose a play, publish poetry which isn't doggerel, and do research ranging from the history of ideas to the technology of coal: such a man must surely be "unsound". But for this prejudice among pedants, Bronowski would surely have been taken more seriously than in fact he was. No doubt there are better coal technologists, better poets, better literary critics, better philosophers, than Bronowski was. The value of his contribution to contemporary thought rests upon his capacity to see the relationships between these disciplines, to create patterns in which the disciplines are intertwined. This is what he did in *Science and Human Values* and in *The Identity of Man*. (It is significant that both these books are based on lectures he gave in the USA: Americans are more hospitable to Renaissance Men than the British are.) And his television series, *The Ascent of Man*, was a heroic attempt to achieve his ambition: "to create a philosophy for the twentieth century which shall be all of one piece". He drew his viewers into participation over questions which have perplexed intellectuals for a millennium.

Anyone who writes and talks as fluently as Bronowski did is saturated with invitations to contribute articles and to give lectures. Characteristically, Bronowski generously accepted many of these invitations and took immense pains to make his contributions elegant and polished. In *A Sense of the Future*, nineteen of these, covering the years 1952 to 1974, have been selected and edited for publication. They comprise his reflections on science. Another volume is promised, which will contain his reflections on aesthetics and literature.

The themes of these essays are familiar to those who have read Bronowski's books. He generates a



Jacob Bronowski

stream of traffic across the bridge between the Two Cultures (a bridge which, oddly enough, C. P. Snow seems never to have discovered). In science, he says, the experiment conceived in imagination is tested by confronting it with physical experience; in literature, the imaginative conception is tested by confronting it with human experience. Experiments and conceptions which survive become part of the cultural heritage. In science, as in humanism, excellence depends on the quality of imagination. In the pursuit of science Bronowski sees also the recipe for ethics; for to pursue science is to search for truth, and no one can verify for himself all the truths he needs to know. Therefore, he has to trust the word of other people. Therefore, the principle of truthfulness "binds society together, because without it the individual would be helpless to tell the truth from the false"; it is a cement to hold society together. So his "social axiom" is that we ought to act in such a way that what is true can be verified to be so.

The most thoughtful essays in the book are about evolution. Bronowski expounds with admirable clarity the

difference between the 'closed' teleology of (say) a watch, whose purpose is implicit in the way its parts are put together, and the 'open' teleology of evolution, where there is selection for 'fitness for change' to meet unpredictable changes in the environment. Of evolution, Bronowski writes, we can say "what Piet Hein said of a work of art, in a penetrating phrase: that it solves a problem which we could not formulate until it was solved".

It is commonly assumed that people who write and lecture fluently possess an inexhaustible reservoir of fresh ideas. This assumption, even for the Bronowskis of this world, is incorrect. Therefore, when they are pressed with invitations to talk here and to write there, they either have to appear churlish, and refuse, or they have to cannibalise some of their own earlier work. So I'm not surprised that there is some repetition in these essays, between chapters 7 and 8, 9 and 10, 12 and 13, 15 and 16. If Bronowski himself had edited these essays, I guess he would have chosen one or the other, not both, of these overlapping pairs. His editors may be criticised for not having done this. I am grateful for the repetitions. They reveal how unsolved problems lodged in Bronowski's mind for years, to be taken out and re-examined from time to time. It is instructive to watch him reflecting on the same subtle idea (for instance, the *Entscheidungsproblem* which he first encountered as an undergraduate in 1931) in two essays, one written in 1966 for the *American Scientist*, and the other eight years later in a symposium on the philosophy of Karl Popper.

Opinions about this book will depend upon the spirit in which it is read. It tackles the most intractable of all philosophical problems: the relation between objective truth as a scientist sees it and subjective consciousness as a man feels it in himself. Those who look for a solution to this problem will, of course, not find it. Those who relish every thoughtful re-examination of this problem will appreciate the elegance with which it is presented. In brief, it is a book to be read not so much for instruction as for delight. □

Lord Ashby is Chancellor of the Queen's University, Belfast, Northern Ireland.

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Point defects in solids

Defects in the Alkaline Earth Oxides. By B. Henderson and J. E. Wertz. Pp. 152 (Taylor and Francis: London, 1977.) £8.50.

THIS monograph is concerned mainly with the structure and properties of point defects studied primarily by the techniques of optical and paramagnetic resonance spectroscopy, a field of investigation to which the authors have themselves made significant contributions during its development over a period of nearly 20 years.

MgO and CaO have been extensively studied, the other oxides less so. Transition metal impurities occupy substitutional sites, sometimes in association with nearby impurity or defect sites, and detailed tabulations of data are given. Whereas early studies were often complicated by the unwanted presence of impurities, it has much more recently become apparent that impurities can play an important part in moderating the production of lattice defects by particle irradiation. The structure of anion vacancy defects

seems to be very well understood, although the interpretation of observations relating to cation vacancy defects still presents some problems. The power of double resonance techniques, both ENDOR and those involving simultaneous irradiation at optical and microwave frequencies, is beautifully illustrated. Compared with the extensive array of experimental results on anion and cation vacancy defects, the information available about point interstitial defects is still sparse.

The appearance of this review is timely as the subject has reached that stage of maturity at which a perspective view of the considerable amount of published work is of real value both as a summary of achievement and as an aid in directing attention to the nature of the most significant problems which remain to be solved. The selection of material is well balanced and concisely presented, and will serve not only as a useful compendium for those engaged in this field but also as a helpful guide and introduction for the newcomer.

T. P. P. Hall

T. P. P. Hall is Lecturer in Physics at the University of Essex, UK.

Electrochemical techniques

Electrochemistry of Biological Molecules. By Glenn Dryhurst. Pp. xii+601. (Academic: New York and London, 1977.) \$47; £33.35.

POLAROGRAPHY was invented in 1922 and soon became an important method of chemical analysis. At the end of the 1950s, there was a decline in the practical everyday use of this method, because direct current (d.c.) polarography, particularly its sensitivity, no longer met the demands then being made on modern analytical methods. Recent years have seen, however, a renaissance in polarographic and voltammetric analyses, and from classical d.c. polarography a number of modern electrochemical techniques have been derived. Of these, derivative (differential) pulse polarography has surpassed d.c. polarography in sensitivity by about two orders of magnitude, and has become increasingly applied to biological research. The publication of Dryhurst's book is therefore timely.

The content of this book covers a narrower research area than that suggested in the title. Of the compounds with biological significance, only nitrogen heterocycles are included. Electrochemistry is mainly limited to the

studies of these substances using polarography and related techniques. The introductory chapter summarises the theory and instrumentation of electro-analytical measurements in a way that will be easily understood even by readers with no experience of electrochemistry. Further chapters contain information on electrochemical reduction and/or oxidation of N-heterocycles, including purines, pyrimidines, pteridines, flavines, pyrroles, porphyrines and pyridines.

About 250 pages are given over to nucleic acids and their constituents. Although the electrochemical reduction of nucleic acid constituents and their analogues has already been studied in considerable detail (there is also quite a vast literature on purine oxidation), more detailed research on the electrochemical behaviour of oligonucleotides is still needed, representing the missing link between studies of polynucleotides and their monomeric units. Nevertheless, research on nucleic acid structure and properties using electrochemical analysis has developed rapidly in recent years. A correlation between the reducibility of nucleic acids in solution and their conformation is the basis for the application of polarographic techniques to the investigation of small changes in DNA double-helical structure. Changes in nucleic acid reducibility can also serve as an indication of conformational changes occurring in nucleic acids adsorbed at interfaces.

On the other hand, literature concerning oxidation of polynucleotides is as yet practically non-existent.

Included in the book are more than 1100 references, from which an impressive amount of electrochemical data has been extracted. This monograph is an invaluable source of information for electrochemists; it will probably also be useful to biochemists

and biologists interested in properties of biologically important N-heterocycles and their analogues.

Emil Paleček

Emil Paleček is Head of the Department of Biophysics of Macromolecules at the Institute of Biophysics, Czechoslovak Academy of Sciences, Brno, Czechoslovakia.

European pollen flora

The North-West European Pollen Flora. Vol. 1. Edited by W. Punt. Pp. 145. (North-Holland: Oxford, New York and Amsterdam, 1977.) \$19.95; Dfl.50.

PALYNOLOGY, the study of pollen grains, is proving to be a valuable technique in a variety of disciplines, particularly environmental ones, both ancient and modern. The recognition of subfossil pollen permits the reconstruction of past vegetation and the detection of palaeo-environmental changes, and the monitoring of present-day pollen fall-out is a necessary part of allergy research. Melissopalynology, the study of pollen in honey, permits the identification of the flowering plants visited by bees.

The rapid growth of these subjects has led to a considerable demand for manuals of palynological techniques and also for data regarding the precise identification of pollen types. At present, the published studies of pollen taxonomic research are widely scattered in the literature and some families have received little attention. This book, and those which will follow it in this series, is designed to remedy this deficiency and to provide an accessible and up-to-date collection of pollen morphological studies. This is being tackled by commissioned review articles which are published in the journal, *Review of Palaeobotany and Palynology*, and which are then collected together in what will be a series of books concerning North-West European pollen.

Obviously, some families are smaller or morphologically less complex than others, so the order in which the published accounts will appear is rather unpredictable. The first collected volume contains accounts of the Caprifoliaceae, Primulaceae, Adoxaceae, Sparganiaceae, Typhaceae, Gentianaceae and Guttiferae. All except for the last of these have been written by members of the Laboratory of Palaeobotany and Palynology of the State University, Utrecht, Netherlands. The account of the Guttiferae is by G. C. S. Clarke of the British Museum (Natural

History).

Each family is divided into a series of "pollen types". The use of the term "type", however, departs from the convention normally adopted in palynological studies, in which "type" is used only when a number of taxa are indistinguishable on the basis of their pollen. Here, a pollen type may contain only a single species which makes the term "type" somewhat redundant. Keys are provided to the pollen types within each family, and full descriptions of the pollen morphology are given. The descriptive terminology of Reitsma is used throughout, which is commendable, for this provides a more consistent and logical system than the earlier ones of Erdtman, and Faegri and Iversen. The descriptions are very full, though it might have been valuable to have added the shorthand designation devised by Iversen and Troels-Smith. Size measurements are given for grains mounted in both glycerol jelly and in silicone oil, which is useful since both of these media are extensively used. One feature which leads to a certain degree of concern is that the descriptions are based upon a very limited number of collections. Some of the types are described on the basis of just one or two collections, and these can hardly be expected to display the full potential range of variation within the taxa.

Excellent collections of photographs are included with each account. These are based upon light field, phase contrast and scanning electron microscopy. They provide extremely valuable supplements to the pollen descriptions. The plates would be far simpler to use if the legends were included on them rather than preceding them. This may seem a minor criticism, but it does limit the speed and efficiency with which the plates can be consulted.

This series will undoubtedly prove to be the definitive work on the pollen of north-west Europe for many decades and one must congratulate the authors, editors and publishers on the production of a most valuable and useful book. I look forward to the appearance of further volumes in this series.

Peter D. Moore

Peter D. Moore is Senior Lecturer in the Department of Plant Sciences, King's College, University of London, UK.

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1977.) At two substantial
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of authors have
this subject in 1977.
by Stonehouse and
very largely with
marsupials, and 28 of the
have studied marsu-
Australia and New Zealand.
the editors' hope that the
"should form an introduction to
research on this long-neglected
fascinating group of mammals"
is a laudable one, only about half the
contributors have attempted to do this
by reviewing their particular field.
These chapters are all useful and some
are excellent.

Clemens' chapter provides an over-
view of current theories of marsupial
origins and relationships to other
therian groups. His conclusion that
most land masses could have been in-
habited by primitive therians in the
early Cretaceous, any one of which
could have been the site of origin of
marsupials and placentals, is provoca-
tive, as it allows for a reversal of the
conventional view of origin in North
America and a southward spread in
the late Cretaceous and Tertiary.

Hayman reviews the field of mar-
supial karyology from a much firmer
data base. The chromosomes of mar-
supials are better known than for most
of the orders of mammals and under-
standing of the evolution has reached
an advanced stage which this chapter
reviews.

The functions of the adrenal cortex
of marsupials are admirably reviewed
by McDonald, while Setchell has
written a wide ranging, scholarly ac-
count of reproduction in male marsu-
pials, a field hitherto often neglected.

Of the ecological chapters, that by
Lee, Bradley and Braithwaite on the
resolution of the unique phenomenon
of synchronous mortality of male
Antechinus is fascinating as a piece of
problem solving by a coordinated group
with several different skills, while
Parker's more theoretical chapter, de-
serves special mention as the first
serious attempt to compare the eco-
logical consequences of marsupial and
eutherian reproductive strategies. Her
conclusion is that the marsupial
strategy allows a much slower rate of
resource utilisation, first by the mother
and subsequently by the slower growing
offspring. "Viewed in this context, the

reproductive biology of marsupials
cannot be deemed less efficient than
that of placentals and may indeed have
considerable advantages in the range of
environments in which marsupials are
found."

This quotation may stand as the
theme of the volume itself, for it is a
conclusion that is reiterated by many
of the contributors whether in relation
to physiology, to biochemistry, to eco-
logy or to behaviour.

I have two criticisms for the editors
of *The Biology of Marsupials*. The first
is that almost half the chapters are
reports of hitherto unpublished work
more appropriate to a journal, or are
narrowly restricted to reviewing an
author's own work. A firmer editorial
direction to authors would have made
the book much more useful and better
value to the wider audience. Second,
those unfamiliar with marsupials are
likely to be confused by the variety of
synonyms and common names used in
different chapters. The worst example

occurs on p328 where four of the eight
species named in Table 19.1 are differ-
ent from the check-list; and the mar-
supial mouse *Antechinus swainsonii*
is provided with the generic name of
the common wombat. Uniform nomen-
clature throughout the volume could
easily have been achieved, since Kirsch
and Calaby in their chapter provide a
comprehensive check-list of all living
species of marsupials.

Despite these shortcomings, *The
Biology of Marsupials* provides many
reasoned and informed reviews, which
will be standard references for some
time to come, and which should help
to lay to rest the old and now unin-
formed view that marsupials are in-
trinsically inferior mammals unworthy
of serious study.

Hugh Tyndale-Biscoe

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Research Scientist in the Division of
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Non-linear acoustics

*Theoretical Foundations of Non-Linear
Acoustics.* By O. V. Rudenko and S. I.
Soluyan. Pp. 274 (Plenum: New York,
1977.) \$47.40.

NON-LINEAR ACOUSTICS deals with a
very wide range of fascinating phenom-
ena nowadays, including ultrasonic pro-
pagation, cavitation in liquids and large
amplitude stress waves in solids, as well
as the more familiar finite amplitude
waves in gases and liquids. Despite the
rapidly increasing practical applicability
of these phenomena, and despite the
review articles, it is a subject which
still receives far too little attention in
the West, where most of the activity is
motivated only by applications to
underwater sonar equipment, to pro-
pagation in aeroengine ducts or to
supersonic aerodynamics.

The Physics Department of Moscow
State University, under the late Pro-
fessor R. V. Khokhlov, has contributed
greatly to non-linear acoustics, and has
taken a wider view of the subject than
any other group. The book under
review is by two of Khokhlov's former
students, now distinguished in their
own right, and is devoted largely to
Soviet work. Some of the work is in
fact new, and much of it is made
accessible to Western readers for the
first time in this publication.

The book gives an account of theo-
retical developments in plane-wave pro-
pagation, with diffusion and relaxation,
of cylindrical and spherical waves, of
sound-sound interaction, acoustic

streaming, the propagation of non-
linear beams with lateral diffraction
included, and of phenomena occurring
in plane waves subject to stochastic in-
fluences of various kinds. Of these, the
last two are the most novel and repay
careful study. Throughout, the authors'
aim is to arrive quickly, though often
superficially, at a relatively simple gov-
erning equation-generalising the well-
known Burgers' equation for plane
flow, which is then solved by *ad hoc*
approximate techniques or numerical
methods. No attempt is made to use
modern asymptotic techniques (such as
have been used in Western work on
non-linear gas dynamic problems,
which are ignored in the references
given here). The book therefore loses
in the range of problems which can be
solved and to some extent in unity of
approach. Comparisons with experi-
ment are only made in a generalised
fashion.

Despite these criticisms this is a
valuable book, and should arouse much
interest in the West. The price, how-
ever, is high and the translation literal
and unidiomatic. More notes from the
translator would have helped; readers
will search in vain for a description of
the "Method of Stagewise Simplifica-
tions", and there is much more here in
similar vein. But Dr Beyer and the
publishers have served us nonetheless
by making this book available reason-
ably quickly after its appearance in
the USSR.

D. G. Crighton

*D. G. Crighton is Professor of Applied
Mathematics and Head of the Department
of Applied Mathematical Studies at the
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APPOINTMENTS VACANT

KING'S COLLEGE HOSPITAL MEDICAL SCHOOL (University of London)

There is a vacancy for a Scientific Officer (Basic grade) in the Department of Medical Microbiology, King's College Hospital, Denmark Hill, London S.E.5. The Department is a combined Public Health Laboratory Service/University/Hospital laboratory. Suitable qualifications would be either a Ph.D. or the Special Examination of the I.M.L.S. Candidates should have 2-3 years post-qualification experience and preference will be given to those with training in electron microscopy. Duties will include running a diagnostic electron microscopy service and research and development in clinical virology. Whitley Council terms and conditions of service will apply. The Department may be visited by arrangement with Professor J. R. Pattison (01-274 6222 ext. 2480) from whom application forms may be obtained. Closing date for applications is February 23, 1978. 1333(A)

THE UNIVERSITY OF LANCASTER DEPARTMENT OF PHYSICS

A Postdoctoral Fellow is required to join a group working on the BIOPHYSICS of synthetic membranes under the direction of Professor R. H. Tredgold. The person appointed will make use of techniques currently under development to form bilipid layers on solid substrates. It is intended to form layers containing various active bio-molecules and to study their electrical and optical properties.

Applicants should be graduates in biochemistry, biophysics or biology and should possess, or be about to obtain a Ph.D. in a relevant field. The appointment is for two years.

Salary £3,761 to £3,975.

Further particulars may be obtained (quoting reference L23/D) from the Establishment Officer, University House, Lancaster LA1 4YW to whom applications (five copies) naming three referees should be sent not later than March 1, 1978. 1314(A)



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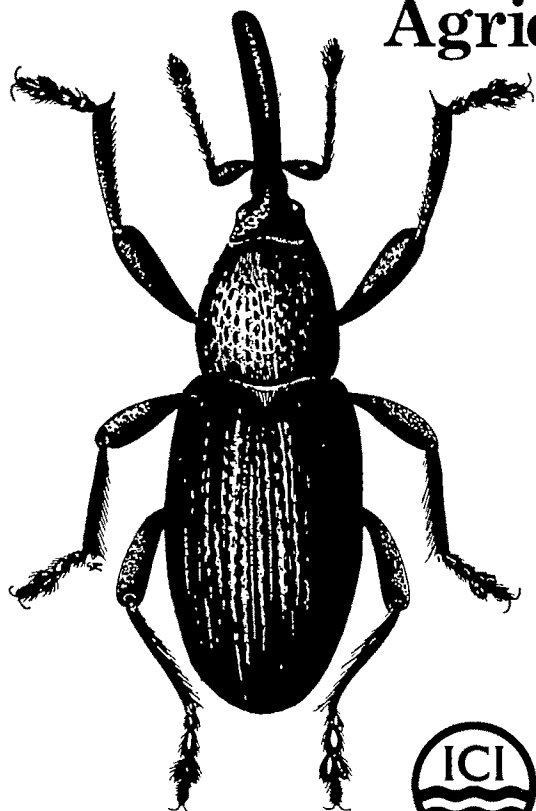
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1364(A)



Agricultural & Public Health Entomologists

Plant Protection Division of ICI is seeking Entomologists to join its international technical development teams. They will be responsible for identifying short and long term product opportunities and bringing innovative thinking based on sound technical knowledge to the expansion of the Group's range of agricultural and public health insecticides. Duties will include providing technical advice to marketing colleagues, customers and research organisations and the organisation and control of field trials programmes.

The posts will be based either at the Divisions Headquarters at Fernhurst, near Haslemere, Surrey, or at its Research Station at Jealott's Hill near Bracknell, Berkshire. Extensive overseas travel will be an essential part of achieving the job objectives.

Successful candidates are likely to have degrees in Entomology with experience in agricultural or public health entomology. Preference will be given to candidates with overseas and/or industrial experience and with linguistic ability (preferably Spanish or Portuguese).

Salaries will be fully commensurate with the qualifications, experience and job responsibilities of the successful applicants, and fringe benefits will include membership of the ICI Profit Sharing Scheme and Pension Fund. Staff Restaurants and Recreation Clubs are run in each location.

For an application form please write or telephone: Mr. H.R.A. Brown, ICI Plant Protection Division, Jealott's Hill Research Station, Bracknell, Berkshire. Telephone: Bracknell 24701.



Plant Protection Division

1355(A)



Wellcome

Technical Officer

to manage an Electron Microscope Unit Bacteriology R & D Department

The Bacteriology Research & Development Department of The Wellcome Research Laboratories (Beckenham) wish to appoint, at a senior level, a Technical Officer who will be responsible for the daily routine operation of the Electron Microscope Unit, including equipment and staff.

The Electron Microscope Unit services the needs of a multi-disciplinary complex of research activities. Its facilities include both transmission and scanning microscopes as well as auxiliary equipment such as a shadowing and freeze etching plant.

Applicants should be fully conversant with various electron microscope techniques necessary for both transmission and scanning microscopes. The successful applicant will be expected to maintain equipment, accept considerable responsibility and is likely to have had at least five years full-time experience in electron microscopy. The salary will be commensurate with the duties

required, commencing in the range £4054—£5305, with prospects for progression.

The Wellcome Research Laboratories are situated in pleasant parkland surroundings within easy reach of Bromley and Beckenham and about 12 miles from Charing Cross. Conditions of employment are attractive and include four weeks' holiday, pension and sick pay schemes, a subsidised canteen and excellent sports and social facilities. Assistance with re-location will be granted where appropriate.

Please write giving brief details of qualifications and experience to R.V. Sutton Personnel Officer, The Wellcome Research Laboratories, Langley Court, Beckenham, Kent BR3 3BS, quoting reference no. U/845.

(1371)A



Research in Therapeutic Applications of Immunology

The Pharmaceuticals Division of ICI is currently reorganising its research programme in applied immunological research. We wish to recruit a scientist with significant experience in immunology to join the present multi-disciplinary immunology group. It is anticipated that this post would be attractive to an immunologist whose research ideas require the support of chemistry, biochemistry and virology. The successful applicant, male or female, will be expected to assume a major innovative role in the discovery of therapeutic agents acting by modification of the immune system. Whilst the research programme will be directed towards a specific target the immunology group carries additional

responsibility for advice and training in relation to several other research programmes. Our modern research laboratories are situated in pleasant surroundings but within easy reach of main road and rail routes. Conditions of service, salary and assistance given to married staff in moving house are designed to attract and retain staff of high calibre.

Could you please write, giving details of qualifications and experience to:

Mr. M.F. Losse
Personnel Officer
ICI Pharmaceuticals Division
Mereside, Alderley Park
Nr. Macclesfield, Cheshire

1345(A)



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Commencing salary will be within the lower part (£3,333-£4,190) of the Lecturer scale except in cases of secondment.

Further particulars and applications forms are obtainable, by postcard request please, from The Personnel Manager, (S2), The Open University, P.O. Box 75, Walton Hall, Milton Keynes, MK7 6AL. Telephone Milton Keynes 63868/9. (There is a 24-hour telephone answering service.) Please state clearly in which post you are interested.

Closing date for applications:
24th February, 1978. 1358(A)

UNIVERSITY OF EDINBURGH DEPARTMENT OF HUMAN GENETICS RESEARCH FELLOW

Postdoctoral biochemist required to work on a project concerned with investigating human fetal-specific and tissue-specific proteins. The appointment will be for one year in the first instance, on the scale £3,333 to £4,190 p.a., but it may be extended for a further 18 months.

Enquiries and applications, including C.V. and names of two referees, to Dr D. J. H. Brock, Department of Human Genetics, Western General Hospital, Edinburgh (telephone 031-332 2471) by March 10. Please quote Reference 5000. 1362(A)

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Applications are invited from registered medical practitioners, who should also possess a research qualification, for this newly created appointment, the duties of which will comprise 6 sessions per week in the Public Health Laboratory participating in hospital and public health microbiology for the relevant health districts, and 5 sessions per week in the University to include undergraduate and post-graduate teaching (with a substantial contribution to the M.Sc. Medical Microbiology degree course commencing in 1979) and research.

The employing authority will be the Public Health Laboratory Service Board and the salary on the N.H.S. Consultant scale. Other terms and conditions of service generally as for Consultants in the N.H.S. The University appointment will be as Reader or Senior Lecturer, depending on the qualifications and experience of the appointee. It is expected that the South West Thames Regional Health Authority will grant an honorary Consultant contract.

The Public Health Laboratory may be visited by arrangement with the Director, Dr R. Y. Cartwright (0483 66091), and the University by arrangement with the Head of the Department of Microbiology, Professor J. E. Smith (0483 71281, Ext. 687).

Full particulars may be obtained from the Secretary to the P.H.L.S. Board, 61, Colindale Avenue, London NW9 5EQ (01-200 2195), to whom applications (12 copies) should be sent to arrive not later than March 6, 1978, stating date of birth, qualifications, experience and published work, and naming three referees. 1361(A)

THAMES POLYTECHNIC
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TECHNICIANS

Applications are invited from suitably qualified and experienced persons for the following technician posts in the School of Biological Sciences:

1. Animal House Technician, Grade 4, to take responsibility for the day-to-day running of a small animal house. A recently qualified graduate in an appropriate subject and with some relevant work experience would be considered for the post.
2. Plant Physiology Technician, Grade 3, to take responsibility for the preparation of materials for plant physiology work and the care of the School's greenhouses. Applicants should have at least an O.N.D. or equivalent qualifications and three years experience.

For both posts some weekend work is involved.

Salary scales: Grade 3: £2,601 to £2,931; Grade 4: £2,835 to £3,216; including London weighting plus personal allowance (i.e. 5% of gross pay min. £2.50, max. £4.00).

Further particulars and form of application may be obtained from the Taffing Officer, Thames Polytechnic, Vellington Street, London SE18 6PF, to whom completed applications should be returned by February 21, 1978. 1349(A)

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CSIRO
AUSTRALIA

Commonwealth Scientific & Industrial Research Organization

Chief of Division

Division of Irrigation Research
Griffith, N.S.W.

CSIRO has a broad charter for research into primary and secondary industry areas. The Organization has approximately 7,000 employees—2,300 of whom are research and professional scientists—located in Divisions and Sections throughout Australia.

General: Applications are invited for the position of Chief of the CSIRO Division of Irrigation Research which is vacant following the retirement of the former Chief, Mr. E. R. Hoare.

The Division is based at Griffith, in the Murrumbidgee Irrigation Area of New South Wales, and strategically located in relation to the main irrigation areas of South East Australia. These areas produce a wide variety of fruits and vegetables, rice and other grain crops and animal products.

The Division has well-appointed laboratories and facilities and provision for field work on two properties totalling 45 ha. It has a staff of 66 including 23 professional staff.

Duties: Chiefs of CSIRO Divisions are responsible to the Executive for the development, scientific direction, integration and management of research programs.

The Division's present research is mainly in the plant sciences, and covers crop breeding, environmental and biochemical physiology, crop adaptation and management, aquatic weed studies and engineering aspects of intensive crop production. To supplement this, the Executive will expect the Chief to develop a strong program on the management of soils and water.

In terms of industry orientation, the Division is concerned with temperate irrigated row crops. Although this is centred on Griffith, the Chief will be expected to develop a program of general relevance to the major temperate irrigation areas of Australia. The Executive would expect the Chief to develop strong links with external bodies

and with other CSIRO Divisions working in related areas.

The Executive believes that to develop these initiatives a vigorous and innovative approach will be required. Substantial research experience in plant science and/or soils and water is desirable.

Salary: Salary will be the subject of negotiation but will not be less than A\$72,495 p.a.

Tenure: The appointment carries Commonwealth Superannuation privileges subject to normal conditions. The position of Chief is offered for a negotiable term of the order of six years, with subsequent options for a further term, if mutually desired, or for a senior position in the Organization on an indefinite basis.

Further Information: Dr. A. E. Pierce, a member of the CSIRO Executive will be visiting the United Kingdom and the U.S.A. during the periods 3rd-12th April, 13th-21st April 1978 respectively, and will be available for discussions concerning the appointment with applicants and other interested parties. Those interested in discussing the appointment should contact The Minister (Scientific), Australian Scientific Liaison Office, Canberra House, 10-15 Maltravers Street, London, WC2R 3EH, England, or The Counsellor (Scientific), Embassy of Australia, 1601 Massachusetts Avenue, N.W., Washington, D.C. 20036, U.S.A. It may be possible to arrange such discussions at a number of points in the United Kingdom and the U.S.A. Scientists interested in discussing the appointment should ensure that the appropriate overseas office is contacted in ample time to enable suitable arrangements to be made.

Further information on the Division is available on request from the Secretary, CSIRO, P.O. Box 225, Dickson, A.C.T. 2602.

Applications: Applications for the position stating full personal and professional details, the names of at least two referees and quoting reference number 500/332 should reach:

The Secretary,
CSIRO,
P.O. Box 225,
Dickson, A.C.T. 2602,
AUSTRALIA
by 10th March 1978

(1384)A

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- Ph.D. degree and, if possible, M.D.
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1272(A)

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As part of the drive to attract high quality entrants to the engineering profession and to British manufacturing industry, and with the encouragement of the University Grants Committee, the University is introducing from October 1978 a special course designed to appeal to well qualified and highly motivated students and to prepare them for worthwhile careers in manufacturing industry.

Applications are invited from good honours graduates for the post of **COURSE SUPERVISOR** for the new course. In view of the nature and objectives of the course relevant industrial experience and close links with manufacturing industry are essential. It is envisaged that the Course Supervisor will be a qualified engineer but applications from persons otherwise qualified will be considered. Experience as a member of the academic staff of a University is also very desirable.

Salary on a scale rising to circa £8,000 per annum, or possibly at a higher level, with placing according to qualifications and experience. Superannuation benefit.

Application forms and further particulars (quoting 8/78) may be obtained from the Academic Appointments Officer, University of Strathclyde, Royal College Building, 204 George Street, Glasgow G1 1XW, with whom applications should be lodged by February 21, 1978.

1315(A)

THE UNIVERSITY OF LEEDS DEPARTMENT OF MICROBIOLOGY

Applications are invited from Medical Graduates for the post of
LECTURER

in the Department of Microbiology. It is hoped that the successful applicant will be eligible for an Honorary Consultant Contract with the Leeds Area Health Authority (Teaching). Salary will be either on the clinical lecturer scale £3,742 to £7,440 (plus £312 supplement) (under review) or on the scale for lecturers with Honorary Consultant Contract £7,536 (plus £312 and £208 supplements) to £8,322 (plus £178 and £208 supplements) to £9,111 to £10,689 (plus £208 supplement). The department may be visited by arrangement with Professor E. Mary Cooke (Leeds 36171 ext. 41).

Application forms and further particulars may be obtained from the Registrar, The University, Leeds LS2 9JT, quoting reference number 96/8/D. Closing date for applications March 10, 1978.

1328(A)

SWISS FEDERAL INSTITUTE OF TECHNOLOGY (ZURICH) RESEARCH ASSOCIATE OR FELLOW IN BIOCHEMISTRY

Applications are invited for the post of Research Associate or Fellow to work on a project concerning protein sequence determinations. Experience in biochemistry, especially in the techniques of protein sequence determination is essential. A Ph.D. or equivalent is preferable. The project is supported for 2 years by a grant of the Swiss Federal Institute of Technology. The salary will be SFr. ~40,000.-/year (S ~20,000.-/year, £ ~15,000.-/year).

Applications, including the names of two referees, should be sent to Professor Dr K. H. Winterhalter, Labor für Biochemie I, Universitätsstr. 16, 8092 Zurich, from whom further details may also be obtained. Please quote Reference 1308 (A).

Biochemical Toxicologist

Occupational Medicine Laboratory £6,310-£8,000

The Occupational Medicine Laboratory at Cricklewood plays an important role in industrial safety by providing a biological monitoring service for industrial exposure to toxic materials, and by undertaking research and development into new monitoring methods. The laboratory has the benefit of an extensive range of equipment including computerised GC-MS.

This post requires a scientist with experience in the study of toxicological mechanisms to carry responsibility for day-to-day management of the laboratory and help the Deputy Director in the development of its research programme. In addition, the person appointed will undertake personal research into biotransformations of hazardous chemicals. The expansion of the laboratory's services will be closely linked to the progress made in this research.

Candidates must have had considerable experience of biochemical toxicology at responsible levels and should have a 1st or 2nd class honours degree in biochemistry or another relevant subject. Appointment will be as Principal Scientific Officer with starting salary within the range £6310-£8000. There are prospects to £11500 and a non-contributory pension scheme.

For further details and application form (to be returned by 3 March 1978) write to Civil Service Commission, Alencon Link, Basingstoke, Hants RG21 1JB, or telephone Basingstoke (0256) 68551 (answering service operates outside office hours). Please quote ref: S(A)677/2 (1329)A

 **Health and Safety Executive**

THE WELLCOME TRUST

Research Grants for Studies in the History of Medicine

The Wellcome Trustees invite applications for grants from post-doctoral scholars with historical training, who are currently associated with a university department in Britain and actively engaged in independent research.

Fellowships of up to two years will be considered to enable applicants to undertake a specific research programme related to some aspect of the history of medicine or medical science. By arrangement with the appropriate authorities, applicants with university posts could take a period of research leave and the cost of a temporary replacement be funded by the Wellcome Trust.

Applicants are asked to put forward their case in a letter of not more than two foolscap pages. A curriculum vitae and, in the case of unestablished workers, the name of a suitably qualified person who has agreed to supervise the research, must also be provided.

The closing date for applications is 31st March, and they should be sent to:-

The Grants Section (HOM),
The Wellcome Trust,
1, Park Square West,
London NW1 4LJ.

1389(A)

UNIVERSITY OF MANCHESTER

DEPARTMENT OF PHYSICS

POSTDOCTORAL RESEARCH ASSISTANT

in Electron Scattering

Applications are invited for the above post, which is for 14 months in the first instance, to work in a flourishing and productive group. The project involves the development of a new position-sensitive multidetector system for electrons which will be used in electron spectrometers and should have applications in a wide variety of fields. The salary is on the scale of £3,333 to £5,627 p.a. (under review).

Enquiries for further details and applications including curriculum vitae and the names of two referees to Dr John Comer, Physics Department, The University, Manchester M13 9PL.

1351(A)

UNIVERSITY OF OXFORD

DEPARTMENT OF HUMAN ANATOMY

TWO TECHNICIAN

Grade III Appointments

Salary £2,455 to £2,788 p.a.

Two technicians are required to assist with biological research, a) in the field of embryology: work to include care of lower vertebrates and electron microscopy, b) in the field of neurobiology and nerve connections: work to include preparative techniques for electron microscopy and histochemistry, O.N.C. or equivalent and A levels in appropriate subjects desirable. The posts are for a period ending July 31, 1979. Applications should be made in writing to the Administrator, Department of Human Anatomy, South Parks Road, Oxford, with the names of two referees indicating for which post the application is being made.

1363(A)

MONASH UNIVERSITY

Melbourne, Australia

CHAIR OF PHYSIOLOGY

Applications are invited for a Professor of Physiology to replace Professor A. K. McIntyre, F.A.A., who will retire from the Foundation Chair at the end of 1978. It is expected therefore that the appointee will take up duty early in 1979. Applicants should be physiologists of distinction able to provide leadership and direction in the research and teaching activities of a large department with responsibilities in the Faculties of Medicine and Science. The present strengths in the research and teaching interests of the Department are in neurophysiology, muscle physiology including the physiology of smooth muscle and autonomic nervous system, endocrinology, renal physiology, circulatory physiology, biophysics and neuropharmacology. There are two establishment Chairs within the Department and a Personal Chair held by Professor M. E. Holman, F.A.A. The successful appointee will have the opportunity to act as Chairman in rotation with the present Chairman, Professor R. Porter, F.A.A., from whom detailed information about the department should be obtained.

Salary: \$A31,248 per annum. Superannuation is based on an endowment assurance scheme, the employee and employer contributing 5 per cent and 10 per cent respectively. A pre-clinical loading of \$A2,500 per annum will be paid to a medically qualified appointee.

Information on application procedure and further particulars may be obtained from the Academic Registrar, Monash University, Clayton, Victoria 3168, Australia, or the Secretary General, Association of Commonwealth Universities (Apts), 36 Gordon Square, London WC1H 0PF.

Applications should reach the Academic Registrar, not later than March 31, 1978. The Council reserves the right to make no appointment or to appoint by invitation at any stage.

1382(A)

UNIVERSITY OF SURINAME

FACULTY OF NATURAL RESOURCES

In the FACULTY OF NATURAL RESOURCES of the University of Suriname at Paramaribo, teaching and research positions are available in:

land and water management 1

(branches drainage/irrigation and land reclamation/conservation)

land and water management 2

(branches agrohydrology and soil physics)

Duties include:

- teaching in these subjects or branches thereof;
- in co-operation with colleagues and other experts to evaluate, adjust and develop further the programme of study;
- participation in the preparation and execution of the research programme of the faculty;
- carrying out of organisational activities in the fields of teaching and research.

Applicants should hold the degree of M.Sc. or equivalent with considerable research experience, preferably in the tropics. Possession of a Ph.D. is an advantage, as well as experience in university teaching.

Period of first contract 3 years, whereupon the contract may be extended. Annual salary is the equivalent of US \$10,000 (under review), the contract bonus approximately 25% thereof. On first appointment and on termination the University will pay the air passage and a baggage allowance.

Applications, including the personalia of the candidate and members of his family, a curriculum vitae, copies of relevant diplomas and testimonials, a recent photo, a list of scientific publications (if any), the names and addresses of three references, and a possible date for assumption of duties, should be addressed before 1 March 1978 to the Board of Governors of the University of Suriname, Kleine Waterstraat 8, Paramaribo, Suriname (South 1323(A)

nature

Nature is considering employing a physical scientist to assist Dr Stuart Sharrock, the Physical Sciences Editor. Such a person would have recently received a Ph.D. and would be hoping to develop a much broader appreciation of science. He or she would be expected to help Dr Sharrock in the assessment of manuscripts, work with other members of the *Nature* staff in compiling the various sections of the journal, establish contacts in the scientific community and, on occasions, write short pieces on recent scientific developments.

Write to Editor, *Nature*, 4 Little Essex Street, London WC2 enclosing a c.v. and any other material you consider relevant.

1375(A)

BIOCHIMICA ET BIOPHYSICA ACTA EDITORIAL SECRETARIAT

Applications are invited for a post in the B.B.A. Editorial Secretariat, which is located in Amsterdam. The Editorial Secretary will be one of a small team of biochemists involved in the selection of manuscripts for publication in the world's largest biochemical journal. The work involves the choosing of reviewers for submitted articles, and subsequent correspondence with authors and editors, leading to eventual acceptance or rejection of the manuscripts. The Secretariat also maintains close contact with the journal sub-editing team, and advises on matters such as nomenclature and terminology. They thus play a central role in the quality control of the journal.

Candidates should meet the following requirements:

- a wide knowledge of the biochemical sciences
- 2-4 years of postgraduate research experience
- initiative, decisiveness, tact and an eye for detail
- an excellent command of the English language
- age under 30 and resident in Europe

The job offers modern working conditions including excellent social services and a good salary and bonus system. Holidays amount to 22 working days per year, plus public holidays.

Applications, including curriculum vitae, should be addressed to:

The Personnel Department
Associated Scientific Publishers
P.O. Box 2400
AMSTERDAM
The Netherlands

Further details are available on request.

1373(A)

BACTERIOLOGIST HEAD OF LABORATORY

An opportunity exists to join a team of microbiologists engaged in chemotherapeutic evaluation of new drugs.

We seek an applicant with experience in bacteriology and perhaps chemotherapy. Preferred age 28-35 years. His (her) main responsibilities will be the development of new model infections, as well as the performance of efficacy and pharmacokinetic studies.

Our research is founded on an interdisciplinary approach, and successful applicants would have the opportunity to pursue basic research projects in well established research groups studying a wide range of biological problems.

We can offer excellent working conditions in well equipped laboratories. An attractive salary will be paid, and travel expenses from country of origin will be refunded.

Interested scientists are invited to send applications stating age, citizenship, qualifications and experience to Dr N. Zacherl, Personnel Manager, Sandoz Forschungsinstitut, A-1235 Wien, Brunner Strasse 59, Austria.

1346(A)

University College Hospital
Gower Street, W.C.1

Department of Obstetrics

Graduate Biochemist

Required for research work on the role of Hyperprolactinaemia in infertility.

The work involves setting up methods to measure Catecholamines by radio enzyme methods and Serotonin by Fluorimetry.

Appointment for one year in the first instance.

Starting salary £3,090 to £4,148 per annum depending on relevant postgraduate experience.

Application forms from Personnel Department
Tele: 01-387 9300 Ext 381 quoting ref: N/GW/AD

1360(A)

THE BRITISH COUNCIL

invites applications
for the following posts:

TEACHER OF
SILVICULTURE
TEACHER OF
BOTANY
(Iran)

Higher School of Natural Resources,
Gorgan

To teach up to B.Sc. level, give practical instruction, set and mark examinations, assist with curriculum development.

Qualifications: Degree plus post-graduate diploma, or preferably M.Sc., in forestry and/or related subjects and at least two years' relevant teaching experience. Men only, single or married candidates, maximum two children under five.

Salary: £5,048 to £6,180 per annum, tax-free.

Benefits: Personal and children's allowances, free furnished accommodation. Two year contracts.

Return fares are paid. Local contracts are guaranteed by the British Council. Please write briefly stating qualifications and length of appropriate experience and quoting reference number 77 HO 40-42 and title of post for further details and application form to The British Council (Appointments), 65 Davies Street, London, W1Y 2AA.

1386(A)

AGRICULTURAL RESEARCH COUNCIL POULTRY RESEARCH CENTRE

Applications are invited for a vacancy at the above Institute for a Higher Scientific Officer/Senior Scientific Officer. The person appointed will participate in an ongoing research programme in the Reproductive Physiology Section. Initially, the work will be concerned with the way in which lipoprotein is transported and partitioned between the various tissues of the body in laying fowls and other poultry and whether or not the mechanisms are associated with the differences in egg-laying characteristics.

Qualifications: 1st or upper 2nd class honours degree in biochemistry, physiology or an appropriate biological science and preferably a Ph.D. Experience in membrane and/or lipoprotein metabolism at the cellular level would be an advantage.

At least two years postgraduate experience is required for appointment at H.S.O. level and 4 years for the S.S.O. grade.

Salary: H.S.O. £3,745 to £4,976; S.S.O. £4,707 to £6,300, including current pay supplements. Non-contributory super-annuation scheme.

Application forms are available from the Secretary, Poultry Research Centre, West Mains Road, Edinburgh EH9 3JS. The closing date for applications is Tuesday, February 28.

1368(A)



Sharpes

LABORATORY TECHNICIAN—PLANT PATHOLOGY

We require a Laboratory Technician to take charge of a Pathology Laboratory under the direction of our Plant Breeders who are engaged in the development of a wide range of crops.

Applicants should have appropriate experience and possess a relevant technical qualification.

Duties include the maintenance of plant disease cultures, assisting with the testing of our breeding stocks for disease resistance, co-operating with our Satellite Seed Testing Station on assessment of disease infection in seed samples and some cytology. A fair amount of glasshouse and field work will be involved.

Remuneration will be based on age and experience. There is a contributory pension scheme.

Apply with detailed curriculum vitae to:—

The Secretary,
CHARLES SHARPE & CO. LTD.
Sleaford, Lincs.

1365(A)

The Daresbury Laboratory has a vacancy at Higher Scientific Officer level for research into the

THEORY OF ATOMIC AND MOLECULAR PROCESSES

The fields of interest of the Theory Group at Daresbury are atomic and molecular, solid state and nuclear.

At the Laboratory a major nuclear structure facility is under construction and work will soon begin on a purpose-built X-ray and ultraviolet source. An IBM 370/165 computer is installed at the Laboratory, and a computational science group has recently been established working mainly in the quantum chemistry area.

The appointment would be a permanent one at Higher Scientific Officer level made according to age, qualifications and experience in the range £3,245 to £4,454. There are also pay supplements of (a) £313.20 per annum and (b) 5% of total salary subject to a minimum of £130.50 per annum and a maximum of £208.80 per annum. There is a non-contributory superannuation scheme and a generous leave allowance.

Applicants, male or female, must possess a good degree in an appropriate subject plus two years relevant experience, and preferably a Ph.D. in the area of atomic and molecular theory or expect to obtain one in 1978.

Closing date: 21 February 1978.

Please write enclosing curriculum vitae, the addresses of two referees and quoting reference number DL/608/T to:

The Personnel Officer

DARESBUURY LABORATORY
Science Research Council
Daresbury, Warrington WA4 4AD

975(A)

MEDICAL RESEARCH COUNCIL LABORATORY ANIMALS CENTRE

Carshalton, Surrey

Applications are invited for the post of Senior Research Officer in the Director's Department.

The successful candidate will be expected to assist the Director with:

- (i) personal assistance with his administrative duties
- (ii) a research programme designed to evaluate specific pathogen free cats.

It is hoped that he/she will be a mature person with an interest in scientific administration. Applicants should have a degree in biological science, together with a Ph.D., and previous experience with small animals would be an advantage although not essential. The post offers excellent conditions of service. Salary in the range £3,999 to £5,199 according to age, qualifications and experience, plus £354 London Weighting, plus £312 Pay Supplement, plus Variable Pay Supplement. Contributory Superannuation Scheme.

Applications, together with a curriculum vitae and the names of two referees should be sent to the Director, Medical Research Council Laboratory Animals Centre, Woodmansterne Road, Carshalton, Surrey SM5 4EF.

1377(A)

MRC

Medical Research Council

QUALIFIED TECHNICIAN (H.N.C. or equivalent)

required for research project in Clinical Science Laboratories, for up to three years from April 1, 1978, to study nutritional and biochemical aspects of vitamin K in man. Previous experience in the techniques of lipid biochemistry or organic chemistry desirable but not essential. Further details from Dr M. J. Shearer, Clinical Science Laboratories, Floor 18, Guy's Tower, Guy's Hospital, London Bridge SE1 9RT.

Salary for technician qualified as above up to £3,275 plus £354 London Weighting.

Apply in writing, stating age and giving details of qualifications and experience, to the Secretary, Guy's Hospital Medical School, London Bridge SE1 9RT, quoting Ref. H.M.

1359(A)

MONTANA STATE UNIVERSITY VISITING FACULTY POSITION(S)

One-year, part-time appointment beginning September 1978. Ph.D. or equivalent required. Particularly suitable for faculty on sabbatical leave. Some teaching required. Extensive research facilities. Outstanding outdoor recreational opportunities in scenic Rocky Mountain setting. Detailed curriculum vitae, list of publications and at least three letters of reference addressing both research and teaching abilities and accomplishments should reach Patrik Callis, Chemistry Department, Montana State University, Bozeman, Montana 59717, U.S.A. (406-994-4801) by March 15, 1978. An equal opportunity/affirmative action employer.

1367(A)

UNIVERSITY OF SURINAME FACULTY OF NATURAL RESOURCES

In the FACULTY OF NATURAL RESOURCES of the University of Suriname at Paramaribo, teaching and research positions are available in:

hydrology (water resources engineering)
and
rural home economics

Duties include:

- teaching in these subjects or branches thereof;
- in co-operation with colleagues and other experts to evaluate, adjust and develop further the programme of study;
- participation in the preparation and execution of the research programme of the faculty;
- carrying out of organisational activities in the fields of teaching and research.

Applicants should hold the degree of M.Sc. or equivalent with considerable research experience, preferably in the tropics. Possession of a Ph.D. is an advantage, as well as experience in university teaching.

Period of first contract 3 years, whereupon the contract may be extended. Annual salary is the equivalent of US \$10,000 (under review), the contract bonus approximately 25% thereof. On first appointment and on termination the University will pay the air passage and a baggage allowance.

Applications, including the personalia of the candidate and members of his family, a curriculum vitae, copies of relevant diplomas and testimonials, a recent photo, a list of scientific publications (if any), the names and addresses of three references, and a possible date for assumption of duties, should be addressed before 16 March 1978 to the Board of Governors of the University of Suriname, Kleine Waterstraat 8, Paramaribo, Suriname (South America).

1322(A)

UNIVERSITY OF LONDON CHAIR OF PHARMACOLOGY AT UNIVERSITY COLLEGE LONDON

The Senate invite applications for the above Chair and the Headship of the Department of Pharmacology at University College London. Initial salary to be agreed, but not less than £8,106 plus £450 London Allowance. Applications (10 copies) must be received not later than March 16, 1978 by the Academic Registrar. (N) University of London, Senate House, London WC1E 7HU, from whom further particulars should be first obtained.

1313(A)

UNIVERSITY OF NOTTINGHAM DEPARTMENT OF CHEMISTRY Applications are invited for the CHAIR OF INORGANIC CHEMISTRY

which will be filled from the beginning of the session 1978/79 or as soon as possible thereafter.

Salary will be within the professorial range.

Further particulars and forms of application, returnable not later than March 13, 1978, from the Staff Appointments Officer, University of Nottingham, University Park, Nottingham NG7 2RD Ref. No. 554.

1347(A)

THE INTERNATIONAL CENTRE OF INSECT PHYSIOLOGY AND ECOLOGY

Nairobi, Kenya

Applications are invited from suitably qualified candidates for the following vacancies.

RESEARCH SCIENTIST (SORGHUM SHOOTFLY PROGRAMME)

REF. SCI/77/12

Qualifications

Ph.D. with five or more years' post-doctoral experience in insect physiology, especially in endocrinology and eco-physiology. High quality research for Ph.D. and evidence for excellence since doctorate will be a distinct advantage.

Responsibilities

The successful candidate will carry out research in the field of sorghum shootfly and will collaborate with other research scientists in the programme which is mainly concerned with eco-physiological research in aestivation-diapause.

POSTDOCTORAL RESEARCH FELLOW (TERMITE PROGRAMME) REF. SCI/77/13

Qualifications

African Scientist, holding Ph.D. or M.Sc. degree in Zoology (Entomology, Physiology) with interest and practical experience in entomology, physiology and ecology. High quality research for Ph.D. or M.Sc. and evidence of creative imagination, careful observation and dedication to scientific work will be a distinct advantage.

Responsibilities

The successful candidate will be responsible for planning, conducting and evaluating experiments on behavioural physiology in tropical termites (communication, pheromones, orientation, construction etc) with special reference to the role of pheromones in the regulation of termite behaviour and caste differentiation.

Salary

Basic salary according to qualifications and experience but, in any case, will not be less than KShs. 86,400/- per annum for the Research Scientist and 66,000/- p.a. for the Post Doctoral Research Fellow.

Other benefits

These include gratuity, housing allowance, group medical insurance scheme, generous annual leave, travelling fare and transport reimbursement allowance.

Applications, giving detailed information on general education, professional qualifications, experience, marital status, age, present terms of employment (including salary), names and addresses of four referees (including one personal referee), and photostat copies of relevant certificates and diplomas should be addressed to:

The Administrative Manager, I.C.I.P.E., P.O. Box 30772, Nairobi, Kenya

to reach him not later than March 7, 1978. 1369(A)

MAX-PLANCK-INSTITUT FÜR BIOCHEMIE, MUNICH

Department of Connective Tissue Research

PROTEIN CHEMIST

required

Applications are invited for the post of Assistant Scientist to join a group working on the primary structure of connective tissue proteins, particularly those found in basement membranes.

Applicants should have a Ph.D. in chemistry or biochemistry. Postdoctoral research experience in protein chemistry and sequencing methods is an advantage.

Salary according to B.A.T. IIb scales. Initial appointment for 2 years with possibility of a permanent position.

Applications to Professor K. Kühn, Max-Planck-Institut für Biochemie, D-8033 Martinsried b. München, Germany. 1350(A)

NATIONAL UNIVERSITY OF LESOTHO

Applications are invited for the post of PROFESSOR AND HEAD OF THE DEPARTMENT OF CHEMISTRY. Consideration will be given to applicants with specialisation in any branch but the present staff will best be complemented by a physical-organic chemist. Candidates should preferably have an interest in undergraduate teaching as well as in research. The appointee will be expected to assume duties by August 1978. Appointment will be on renewable short-term contract. Salary scale: Professor R8,580 to R9,000 (£1 sterling equals R1.68). The British Government may supplement salary by £3,456 p.a. (sterling) for married appointee and £2,274 p.a. (sterling) for single appointee (reviewed annually and normally free of all tax) and provide children's education allowances and holiday visit passages. Superannuation: Non-contributory for appointees on permanent terms of service. Appointees on short contract terms receive 25 per cent gratuity in lieu of superannuation for the first two years of the contract, rising to 27½ per cent and 30 per cent for each subsequent and similar period of service. 15 per cent inducement allowance for expatriates not qualifying for any supplementation scheme. Family passages, baggage allowance; reasonable rental for accommodation; education allowance for expatriates; vacation and study leave. Detailed applications (2 copies) to be sent with curriculum vitae together with names and addresses of 3 referees to Registrar (Appointments) National University of Lesotho, Roma Lesotho by March 1, 1978. Applicants resident in U.K. should send one copy to Inter University Council, 90/91 Tottenham Court Road, London W1P 0DT. Further details may be obtained from either address. 1357(A)

THE UNIVERSITY OF MANCHESTER LABORATORY TECHNICIAN (Research) Grade 5

Applicants must provide evidence of considerable competence in general and specialised histological techniques and ability to use all forms of modern apparatus is essential. They should have an H.N.C. or equivalent qualification and have had a minimum of 8 years relevant experience including experience in electron microscopy and auto radiographic procedures. The ability to instruct and supervise Junior Technicians is also a requirement. Candidates must be able to develop new techniques and be conversant with general research procedures.

Salary Scale £3,186 to £3,720 p.a. Applications in writing, giving details of age, education and qualifications, together with technical experience and the names of two referees should be made to Professor P. F. Harris, Director of the Anatomical Laboratories, Stopford Building, Oxford Road, Manchester M13 9PT. Closing date Friday, 24th February, 1978. 1383(A)

GUY'S HOSPITAL MEDICAL AND DENTAL SCHOOLS

RESEARCH ASSISTANT (Immunologist/Biochemist)

Pre- or postdoctoral immunologist or biochemist having experience in immunochemical techniques required for a period of two years in the first instance. The person is required to investigate immune responses to Streptococcus mutans in rhesus monkeys.

Salary according to qualifications and experience, with £450 per annum London Allowance and superannuation.

Apply in writing, with curriculum vitae, to the Secretary, Guy's Hospital Medical School, London Bridge SE1 9RT, quoting Ref. I.M.4. 1348(A)

KENYATTA UNIVERSITY COLLEGE—KENYA

Applications are invited for the post of SENIOR LECTURER/LECTURER in the ZOOLOGY DEPARTMENT. Applicants should have Ph.D. in any field of Zoology. Preference will be given to those qualified to teach Invertebrate Zoology, fresh water Biology or fish Biology. Salary Scales—Senior Lecturer £K2,772 to £K3,600 p.a., Lecturer £K1,800 to £K3,096 (£K1 equals £1.35 sterling). The British Government may supplement salaries in range £3,354 to £4,128 p.a. (sterling) for married appointee or £2,184 to £2,880 p.a. (sterling) for single appointee (reviewed annually and normally free of all tax) and provide children's education allowance and holiday visit passages. Terms of service include family passages, superannuation, subsidised housing, medical aid scheme plus various allowances. Detailed applications (2 copies) with a curriculum vitae and naming 3 referees to be sent to Registrar, Kenyatta University College, P.O. Box 43844, Nairobi, Kenya by February 28, 1978. Applicants resident in the U.K. should send one copy to Inter University Council, 90/91 Tottenham Court Road, London W1P 0DT. Further particulars may be obtained from either address. 1356(A)

UNIVERSITY OF ASTON IN BIRMINGHAM DEPARTMENT OF PHARMACY Cancer Chemotherapy Group CONTRACT

RESEARCH FELLOW

Our multidisciplinary group has a vacancy for a POSTDOCTORAL RESEARCH FELLOW, who has a background in biochemistry or biochemical pharmacology, to join the investigation of the effects of certain antitumour agents on folate metabolism. The work is supported by the Cancer Research Campaign with a commencing salary within the range £3,333 p.a. to £3,975 p.a. (under review).

Preliminary enquiries may be made to Dr J. A. Hickman, Department of Pharmacy (Tel: 021-359 3611 Ext. 6066). Application forms and further particulars may be obtained from the Staff Officer, University of Aston in Birmingham, Gosta Green, Birmingham B4 7ET (Tel: Ext. 201) to whom applications should be returned (quoting Ref. No. 249/6) not later than March 3, 1978. 1341(A)

UNIVERSITY COLLEGE LONDON

DEPARTMENT OF CHEMISTRY POSTDOCTORAL

RESEARCH ASSISTANT

required from September 1, 1978 for work in collaboration with Dr T. M. Cresp on the total synthesis of biologically active sesquiterpenes. Salary: £3,569 plus £450 London Allowance: U.S.S. The post is tenable for one year with possible renewal for a second year. Applications, consisting of curriculum vitae and names of two referees, should be sent as soon as possible to Dr T. M. Cresp, Dept. of Chemistry, University College London, 20 Gordon St., London WC1H 0AJ, U.K. 1379(A)

RESEARCH TECHNICIAN (Grade 5)

required in Technical Services Section. Applicants should have some experience of electron-microscopy and be familiar with routine histological techniques. 37½-hour week, Monday to Friday. Pension scheme, excellent working conditions and 4 weeks annual holiday. Salary range £3,651 to £4,185 per annum including London weighting. Applications, stating details of experience and qualifications, should be sent to Mr C. L. Meredith, Departmental Superintendent, Zoology Department, Imperial College, London SW7. 1378(A)

EAST MALLING RESEARCH STATION MAIDSTONE, KENT

Director

Applications are invited for the post of Director of the East Malling Research Station which will become vacant when the present Director retires early in 1979.

The Station, financed by a grant-in-aid from the Agricultural Research Council, is concerned with basic and applied research on the propagation, culture and cropping of fruit and other perennial woody plants, their protection against pests and diseases and post harvest treatment including storage.

Candidates should be eminent in one of the scientific disciplines relevant to the work of the Station, with proven ability in the conduct and management of research and an awareness of the problems of practical horticulture.

The appointment is in the grade of Chief Scientific Officer; salary £11,878 including Phase 2 pay supplement. There is a non-contributory superannuation scheme.

Applications are to be submitted by 28th April, 1978 to the Secretary, East Malling Research Station, East Malling, Maidstone, Kent ME19 6BJ from whom further details are available. 1338(A)

ELSEVIER/NORTH HOLLAND BIOMEDICAL PRESS

have a challenging position available for a

SCIENTIFIC JOURNALIST

to edit a topical magazine in the Biochemical Sciences.

We are looking for a good writer, capable of taking decisions under pressure and working to deadlines. He/she will be expected to liaise with external editors in processing reviews and gathering/rewriting news and views.

An advanced qualification and/or research or teaching experience in the biosciences is preferred, with proven writing ability and possibly with some experience of publishing and editing.

The position will be based in Cambridge but mobility will be a definite advantage as the successful candidate will be expected to spend some time in Amsterdam and to travel to scientific meetings.

Further information on this post may be obtained from Dr. J. Franklin in Amsterdam, telephone 020-5153152. Applications with career details and salary required should be addressed to The Personnel Dept., ASP, P.O. Box 2400, Amsterdam, The Netherlands. 1374(A)

JUNIOR TECHNICIAN

required in Department of Chemical Pathology to assist with routine assay of steroid hormones by radioimmunoassay and with research project on steroid receptors in human endometria. Salary according to age, and qualifications within scale £1,733 to £2,758 p.a. plus £354 p.a. London Allowance. Apply with full details and names of two referees to: The Secretary, St Mary's Hospital Medical School, Paddington, London W2 1PG quoting Ref. JT/CP/N. 1336(A)

UNIVERSITY OF IDAHO

Assistant or Associate Professor/Ornamental Horticulturist. University of Idaho. Responsibilities: approximately 80% teaching, 20% research. Ph.D. required. Position available after July 1, 1978. Position open until acceptable candidate is selected. Inquiries should be sent to: Ornamental Horticulture Position, Dept. of Plant & Soil Sciences, University of Idaho, Moscow, Idaho 83843. (Ph. 208/885-6276. A.A./E.O. employer and educational institution. 1148(A)

UNIVERSITY OF SURINAME FACULTY OF NATURAL RESOURCES

In the Department of Geodesy of the Faculty of Natural Resources of the University of Suriname at Paramaribo, teaching and research positions are available in:

A Photogrammetry and

B Calculus of observations

Duties include:

- teaching in these subjects and in land surveying;
- in co-operation with colleagues and other experts to evaluate, adjust and develop the programme of study;
- participation in the preparation and execution of the research programme of the faculty;
- carrying out of organisational activities in the fields of teaching and research.

Applicants should hold the degree of M.Sc. or equivalent with considerable research experience, preferably in the tropics. Possession of a Ph.D. is an advantage, just as experience in university teaching.

Period of first contract 3 years, whereupon the contract may be extended. Annual salary is the equivalent of US \$10,000 (under review), the contract bonus appr. 25% thereof. On first appointment and on termination the University will pay the air passage and a baggage allowance.

Applications, including the personalia of the candidate and members of his family, a curriculum vitae, copies of relevant diplomas and testimonials, a recent photo, a list of scientific publications (if any), the names and addresses of three references, and a possible date for assumption of duties, should be addressed before 16 March 1978 to the Board of Governors of the University of Suriname, Kleine Waterstraat 8, Paramaribo, Suriname (S. Am.). 1321(A)

ROTHAMSTED EXPERIMENTAL STATION Harpenden, Herts. AL5 2JQ ASSISTANT STATISTICIAN

to analyse data from agricultural and other biological experiments using the Station's computer and to help in the supervision of the data-processing staff. Degree, H.N.C. or equivalent qualification in mathematics or statistics. Some knowledge of agriculture or biology an advantage.

Appointment in grade of Scientific Officer £2,592 to £4,032 including pay supplements. Starting salary according to qualifications and experience. Non-contributory superannuation.

Applications, giving names and addresses of two referees and quoting ref. 350 to the Secretary by March 3, 1978. Further details on request. 1353(A)

TROPICAL POST-HARVEST PHYSIOLOGIST

The United Fruit Co. needs a plant physiologist with specialisation in post-harvest physiology in its research laboratories in Central America.

Prior experience in the tropics and a knowledge of Spanish would be useful but are not essential requirements.

Salary starts at U.S. \$16,000 per annum with low local income tax; pension scheme, health insurance and good local junior schooling coupled with educational assistance for older children; annual home leave of 28 days with family transportation paid; costs of removal of successful applicant will be met by the Company.

Please apply with curriculum vitae and addresses of two referees to: Manager Administration, Tropical Research Division, United Fruit Co., La Lima, Rep. of Honduras, Central America. 1366(A)

Experimental Officer -Drug Metabolism

The Pharmaceuticals Division of ICI has a vacancy for an Experimentalist to work in the Drug Metabolism group of its Safety of Medicines Department. The job holder, male or female, will be responsible for metabolism studies on candidate drugs, but will report to a PhD qualified scientist. Applicants should have experience of liquid scintillation counting and combustion techniques for the assay of labelled drugs in body fluids and in vivo techniques of studying the metabolism of drugs. The successful candidate is also likely to possess a first degree or equivalent in Chemistry.

Our modern research laboratories are situated in pleasant surroundings, but within easy reach of main road and rail routes. Conditions of service, salary and assistance to married staff in moving house are designed to attract and retain staff of high calibre.

Please write giving details of qualifications and experience to, Mr. M. F. Losse, Personnel Officer
ICI Pharmaceuticals
Division
Mereside Research
Laboratories
Alderley Park
Nr. Macclesfield
Cheshire
1344(A)



Higher Scientific Officer £3,254 - £4,454

(plus supplements)

Applications are invited for a pensionable post in the Department of Commerce, Industrial Science Division, which is presently located at 16 Comber Road, Newtownards but will shortly be transferred to Lisburn.

The post is in the Metallurgy and Metallurgical Chemistry section and the duties include providing a rapid technical investigation service to industry in this field from production problems and material selection to component failures. Practical experience of metal failures, welding defects, corrosion, heat treatment, electroplating and chemical analysis of metals using modern instrumental techniques is essential. Experience in Photomicroscopy and Macro photography would be an advantage.

Applicants must be under 30 years of age on 31 December 1978 with a 1st or 2nd class honours degree in Chemistry, Metallurgy or a related subject and at least 2 years' appropriate post graduate experience.

Exceptionally applications may be considered from candidates over the age limits who have specialised experience.

There are excellent promotion prospects to Senior Scientific Officer (£4,185-£5,778) and Principal Scientific Officer (£5,514-£7,205). Starting salary will be related to qualifications and experience.

In addition to the salary scales quoted pay supplements of between £310.59 and £522.00 per annum will be payable.

Please write or telephone for an application form, quoting job reference SB 61/78/NA, to the Civil Service Commission, Rosepark House, Upper Newtownards Road, Belfast BT4 3NR (telephone Dundonald 4585 ext 256). Completed forms must be returned to arrive not later than 23rd February 1978. 1326(A)



**NORTHERN IRELAND
CIVIL SERVICE**

THE UNIVERSITY OF MANCHESTER DEPARTMENTS OF SURGERY AND PATHOLOGY

Postdoctoral IMMUNOLOGIST required with experience in the techniques of Cellular Immunology as applied to patients. The post is for one year initially, but renewable. The objective is to study the nature of the immune deficit in injured and malnourished patients. A full-time technician in Immunology and adequate laboratory facilities will be available for the project. Salary scale for Research Staff with starting point up to £4,811 p.a. Enquiries to Professor W. L. Ford, Department of Pathology, Stopford Building, Oxford Road, Manchester M13 9PT or to Professor M. Irving, Department of Surgery, Hope Hospital, Eccles Old Road, Salford M6 8HD. Applications to Professor Irving by February 20. 1327(A)

THE SCHOOL OF PHARMACY UNIVERSITY OF LONDON LECTURER IN PHARMACEUTICAL CHEMISTRY

Applications are invited from suitably qualified persons who have an interest in, and an ability to teach Organic/Inorganic Chemistry. Applicants should have some teaching and research experience, and should normally possess a higher degree.

Normal non-clinical university academic terms of appointment. Point of entry to Lecturer Salary Scale dependent on qualifications and experience.

Applications, by not later than February 28, 1978, in the form of a letter accompanied by curriculum vitae to:-

The Personnel Officer,
The School of Pharmacy,
29/39 Brunswick Square,
London WC1N 1AX,

from whom further information may be obtained. 1312(A)

UNIVERSITY OF BIRMINGHAM DEPARTMENT OF BIOCHEMISTRY

Applications are invited for a post of:

LECTURER IN BIOCHEMISTRY

tenable from October 1978, or earlier.

The successful candidate will be expected to participate in the general and specialised teaching programmes of the Department which provides courses for science, medical and dental undergraduates. Excellent facilities are available for most types of biochemical research.

Salary within the range £3,333 to £4,403 p.a. (plus U.S.S.), depending on age, qualifications and experience.

Applications (3 copies) naming 3 referees should be sent by March 6, 1978 to the Assistant Registrar (Senate), University of Birmingham, P.O. Box 363, Birmingham B15 2TT, from whom further particulars may be obtained. 1311(A)

UNIVERSITY OF NOTTINGHAM DEPARTMENT OF AGRICULTURE AND HORTICULTURE

Applications are invited for a
**LECTURESHP IN
HORTICULTURE**

which will be effective from the beginning of the session 1978/79. Preference will be given to candidates who have specialised in aspects of fruit or vegetable production.

Salary will be within the range £3,333 to £6,655 but the appointment will be made initially within the first three points of the scale (£3,333 to £3,761) per annum.

Further particulars and forms of application, returnable not later than February 28, 1978, from the Staff Appointments Officer, University of Nottingham, University Park, Nottingham NG7 2RD. Ref. No. 555. 1318(A)

MARINE SCIENCES RESEARCH LABORATORY MEMORIAL UNIVERSITY OF NEWFOUNDLAND RESEARCH SCIENTIST AQUACULTURE

To conduct research on problems directly related to aquaculture in Newfoundland. The scientists will be expected to assume increasing responsibility for conducting projects already in progress and advise on major changes where appropriate.

A Ph.D. in experimental animal biology. Research experience on live animals is essential. Applicants must be prepared to conduct research in the field as well as in the laboratory. Ability to liaise with non-scientists is important.

A research scientist at the M.S.R.L. enjoys essentially the same privileges as an academic appointment.

The M.S.R.L. is located three miles from St John's and is a modern well-equipped research laboratory.

Salary is commensurate with qualifications and experience.

Please respond with a biographical sketch, list of publications and three references by March 31 to:

Dr D. R. Idler, Director
Marine Sciences
Research Laboratory,
Memorial University
of Newfoundland,
St John's, Nfld.,
Canada A1C 5S7 1385(A)

STATISTICIAN

The Forestry Commission offers a period appointment of 3 years for a Statistician at their Forest Research Station, Alice Holt Lodge, Wrecclesham, Farnham, Surrey.

The successful candidate will work with a small team of Statisticians advising on the design and analysis of R&D projects in Forest Management and Protection; develop mathematical models for problems in protection of forests and trees against pests, work study etc; co-operate with forest scientists to improve the use of statistical methods and write computer programs to implement mathematical models.

Candidates (who should normally be aged under 30 at 31 December 1978) should have a good honours degree in mathematics with statistics or MIS with experience. **Salary:** within the range for Scientific Officer (£2149—£3527) or for Higher Scientific Officer (£3254—£4454) depending on qualifications and experience. Supplements of £313.20 and 5% of total earnings subject to a minimum of £130.50 per annum and a maximum of £208.80 per annum are also payable on each pay scale.

Closing date for applications: application forms and further details may be obtained from Mr C Robbins, Personnel Branch, Forestry Commission, 231 Corstorphine Road, Edinburgh EH12 7AT. Completed application forms should be returned not later than 20 March 1978.

(1380)A

NATIONAL INSTITUTE OF AGRICULTURAL BOTANY CHEMIST/BIOCHEMIST

(Ref. SV/182N)

TECHNICAL OFFICER (Trainee) required in the Institute's Chemistry and Quality Assessment Branch for analytical work on oilseeds. Candidates, preferably under 28 years of age, must have a first or second class honours degree, or equivalent in an appropriate discipline; experience of gas chromatography would be an advantage.

Salary £2,892 to £3,588 including supplements.

Further particulars and application forms from the Establishments Officer, National Institute of Agricultural Botany, Huntingdon Road, Cambridge CB3 0LE. Telephone: Cambridge 76381, ext. 234.

Closing date February 28, 1978.
1387(A)

UNIVERSITY OF ALBERTA

The Department of Geology, University of Alberta, Edmonton, Alberta, Canada T6G 2E3 invites applications for a full-time continuing faculty position in the field of clay mineralogy from July 1, 1978 or as soon as possible thereafter. The preferred applicant should have a mineralogical background and be prepared to apply his researches to local sedimentological problems, and/or the industrial uses of clays. The appointment will be at the Assistant Professor level (current salary level \$17,625 to \$22,888 plus health, pension and insurance fringe benefits).

Applications, with names of three referees, should be sent to the Chairman of the Department, from whom further details may be obtained. Closing date May 31, 1978.

1309(A)

RESEARCH ASSISTANT

required in Department of Experimental Pathology to investigate human papovavirus infection in pregnancy. Post tenable for one year in first instance. Candidates should have a good B.Sc. or M.Sc. in Biology or related science. Experience in Virology, electron microscopy or cytopathology an advantage. Project suitable for thesis or higher qualification. **Salary** £3,377 p.a. including London Allowance. Apply, The Secretary, St Mary's Hospital Medical School, Paddington, London W2 1PG quoting Ref. RA/EPC/N. 1335(A)

FREIE UNIVERSITÄT BERLIN

Klinikum Steglitz FB 2 WE 05

IMMUNOLOGY

RESEARCH UNIT

POSTDOCTORAL RESEARCH ASSISTANT AH 1

Applications are invited for the above position which will begin on April 1, 1978. The applicant with several years experience in Immunology will join the group of Professor Diamantstein working on the mechanism of lymphocyte activation and on regulation of the immune response.

Salary approximately £8,000 to £8,500 p.a. (minus tax) depending on experience, age, family status. Applications to: Professor Diamantstein, Klinikum Steglitz, Freie Universität Berlin, Hindenburgdamm 27, 1000 Berlin 45, Germany. 1324(A)

CHRISTIE HOSPITAL AND HOLT RADIUM INSTITUTE, PATERSON LABORATORIES

A limited number of appointments will be available from October 1, 1978 for full-time studies leading to higher degrees at the University of Manchester. The selected areas of cancer research are concerned with biochemistry, cell biology, cytology, haematology and immunology.

Applicants should have a good Honours degree in science or hope to obtain a First or Upper Second Class degree in 1978.

Remuneration will be at the rates applicable to postgraduate students supported by the Medical Research Council (M.R.C.).

Applications should be sent to Professor L. G. Lajtha, Paterson Laboratories, Christie Hospital & Holt Radium Institute, Withington, Manchester M20 9BX. Ref. 78/6. 1316(E)

MISCELLANEOUS

BRITISH FOUNDATION FOR AGE RESEARCH invites

Research Projects in Incontinence of the Elderly, for which funds are available. Closing date Friday, March 10, 1978

Details and Application Forms from the Director BFAR, 101, Queen Victoria Street, EC4P 4EP. 1372(J)

Director

of the Poultry Research Centre, Edinburgh

Applications are invited for the post of Director of the Agricultural Research Council's Poultry Research Centre which will become vacant when the present Director retires on the 27th June, 1978.

The work of the Centre is concerned with research on domestic poultry and their environmental needs which include work in the fields of physiology, anatomy, nutrition and behaviour much of which involves interdisciplinary collaborative research.

Candidates should have qualifications or equivalent experience in one or more of these subjects together with a proven ability in the conduct and management of research. Preference will be given to candidates with relevant experience of poultry research.

The post of Director is graded Deputy Chief Scientific Officer, the initial point on the salary scale being £10,180 rising by two annual increments to £11,190. In addition a Phase II supplement of £208.80 per annum is currently payable. There is a non-contributory pension scheme.

The closing date for applications is 27th February 1978. Further particulars and application forms may be obtained from Dr. Gareth M. Price, Agricultural Research Council, 160, Great Portland Street, London, W1N 6DT. 1337) A



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CONFERENCES

EUROPEAN TISSUE CULTURE SOCIETY

Glasgow, July 3-5, 1978

Main topics—Differentiation in Friend cells and Teratocarcinoma, Somatic Cell Genetics, Characterisation of Tumour Cells in vitro, plus workshops and poster sessions on a wide range of topics related to tissue culture.

Inquiries to: R. I. Freshney, Beatson Institute, Garscube Estate, Bearsden, Glasgow, Scotland. 1319(C)

12th International Leukocyte Culture Conference

June 25-30, 1978

Ben Gurion University, Beer Sheva, Israel

Theme: Cell Biology and Biochemistry of Leukocyte Function. Topics include, leukocyte membranes; cytoskeleton; lymphocyte activation; thymic factors; lymphokines; cytotoxicity; leukocyte killing; genetics; intercellular co-operation; viruses, radiation and carcinogenic agents; application to clinical medicine. Registration forms and information will be sent on request. Professor M. R. Quastel c/o Secretariat, P.O.B. 16271, Tel Aviv, Israel. 1320(C)

COURSES

UNIVERSITY OF LONDON KING'S COLLEGE NEW INTERCOLLEGIATE COURSE

starting October 1978

M.Sc. IN HUMAN AND APPLIED PHYSIOLOGY

A one-year course to train graduates in measuring and studying the physiological functioning of man in normal and abnormal environments. The course would equip students to specialise in the applications of physiology to man in industrial, service and clinical situations and the teaching of human physiology. Topics include: basic measurements; life support systems; exercise; locomotor; postural; climatic; high altitude and hyperbaric physiology. Applicants should normally have a good degree in Physiology or be medically qualified.

Further details and application form from Dr S. Rowlands, King's College, Strand, London WC2R 2LS, quoting reference 191602/N.

Closing date for applications: March 31, 1978. 1388(D)

THE UNIVERSITY OF MANCHESTER

M.Sc. IN OCCUPATIONAL HYGIENE

(by part-time study)

Held on two days a week, this two-year course comprises four terms of formal teaching sessions, covering the social, scientific and engineering aspects of occupational hygiene and nine months of research study aimed at providing first hand experience in the evaluation of the working environment. The course is open to science or engineering graduates currently employed as occupational hygienists. Further details from Dr F. F. Cinkotai, Department of Occupational Health, Stopford Building, The University, Manchester M13 9PT. 1340(D)

FELLOWSHIPS

UNIVERSITY COLLEGE
LONDONDEPARTMENT OF CHEMISTRY
POSTDOCTORAL FELLOW

Applications are invited for a post-doctoral fellowship for work on the application of microwave spectroscopy to the study of simple hydrogen bonded complexes, in collaboration with Professor D. J. Millen and Dr A. C. Legon. Applications, including curriculum vitae and names of two referees, should be sent to Professor D. J. Millen, Department of Chemistry, University College, 20 Gordon Street, London WC1H 0AJ. 1354(E)

UNIVERSITY OF
BIRMINGHAMDEPARTMENT OF PHYSICS
RESEARCH FELLOWSHIP

Development of well-logging instrumentation

Applications are invited from Physicists or Electrical Engineers with research experience to design and develop a prototype Nuclear Magnetic logging device for measuring the porosity and permeability of rocks penetrated by water wells. The project will involve close collaboration with the Hydrogeological and Geophysics sections of the Department of Geological Sciences and with the Hydrogeology Division of the Institute of Geological Sciences. Salary (under review) in the range £3,333 to £5,627, plus superannuation; maximum starting salary will be £3,975, tenable from April 1, 1978 for one year with probable extension of one year.

Applications (3 copies) including full curriculum vitae and the names of three referees should be sent to the Assistant Registrar, Science and Engineering, University of Birmingham, P.O. Box 363, Birmingham B15 2TT, by Friday, February 17, 1978.

Please quote ref: ND4. 1332(R)

UNIVERSITY OF SYDNEY
POSTDOCTORAL

RESEARCH FELLOWSHIPS

Applications are invited for the above-mentioned awards from candidates who hold a Ph.D. degree or have research publications revealing equivalent status.

The Fellowships are not intended for persons who are in, or have occupied a career position since being awarded the Ph.D.

The Fellowships, which are tenable in any field, are valued at between \$A12,872 and \$A14,286 p.a. and provide return fares. They are tenable for one year with the possibility of renewal for a second year.

Further information is available from the Registrar, University of Sydney, NSW 2006, Australia, with whom applications close on 1 May 1978. 1381(E)

NATIONAL INSTITUTES
OF HEALTHVISITING FELLOWSHIP IN
BIOPHYSICAL CHEMISTRY

Applications are invited for a post-doctoral fellowship of one to two years duration for development of novel methods of measuring the oxygenation of haemoglobin and the aggregation of sickle haemoglobin.

Applicants should be non-U.S. citizens with less than two years post-doctoral experience. Starting salary \$10,000 to \$10,800 plus benefits.

Applications (with curriculum vitae) or letters of inquiry should be addressed to Dr A. P. Minton, Laboratory of Biophysical Chemistry, N.I.A.M.D.D.-N.I.H., Bethesda, Md. 20014, U.S.A. 1310(E)

UNIVERSITY OF SURREY

DEPARTMENT OF
METALLURGY & MATERIALS
TECHNOLOGY

Applications are invited for the post of Redland Research Fellow tenable in the Department of Metallurgy and Materials Technology at the University of Surrey.

The research programme carried out by the Fellow will relate closely to the research and development interests of Redland Limited, and the Fellow normally carries out a number of subsidiary investigations requested by the Company's Research and Development Centre. The post affords an excellent opportunity for the incumbent to familiarise himself with industrial research and to make the transition from academic to the industrial environment.

Applicants should have a Ph.D. or equivalent experience in some area of material science, or solid state chemistry. Preference may be given to candidates with interests or experience in silicate chemistry, Portland cement and ceramics. In view of the nature of the appointment, candidates having the ability to formulate new experimental approaches to unresolved problems would be preferred. The Fellow appointed will undertake research in materials of interest in construction and building and the main concern will be with property structure relationships of interest in developing new or improved materials.

The appointment would be made for two years in the first instance, renewable thereafter on a yearly basis.

Remuneration will depend on age and experience and will be in the range £3,333 to £3,761 (under review), on scales related to the University lecturers' scales. Applications, from men and women, in the form of a curriculum vitae, with the names and addresses of three referees, should be sent to:

Professor J. E. Bailey,
Department of Metallurgy and
Materials Technology,
University of Surrey,
Guildford, Surrey GU2 5XH. 1317(E)

SYMPOSIUM

LEUKAEMIA RESEARCH FUND

International Research Symposium on

"BONE MARROW TRANSPLANTATION"

will be held at the Royal College of Physicians, London, on Monday, May 10, 1978. Registration fee (includes lunch) £6. Apply for details and registration form to:

Leukaemia Research Fund,
43 Great Ormond Street,
London WC1N 3JJ.

Tel: 01-405 0101 1342(M)

AWARDS

SASKATCHEWAN SCHOLARS PROGRAM

The Saskatchewan Research Scholar Awards are to encourage and assist researchers who have demonstrated high promise in research and scholarship. Scholars receive \$17,000.00 per year for two years, possible renewal for a third year. Ph.D. (or equivalent) and normally one or two years additional experience, plus the ability to do independent research. Scholarship may be held in any field, Humanities, Social Sciences, Life Sciences, Physical Sciences or Engineerings. Canadian citizens and landed immigrants have priority. Closing date for application May 15, 1978 for awards to commence between July 1, 1978 and January 1, 1979.

Further information and application forms available from The Dean, College of Graduate Studies and Research, University of Saskatchewan, Saskatoon, Saskatchewan, Canada S7N 0W0. 1331(N)

STUDENTSHIPS

SIR WILLIAM DUNN
SCHOOL OF PATHOLOGY

University of Oxford

RESEARCH STUDENTSHIP

Applications are invited from students expecting to graduate in 1978 with a good honours degree in biochemistry or chemistry and wishing to undertake research (leading to the degree of D.Phil.) on the biochemistry of penicillinases.

The studentship's value is at the level of a state grant and includes the payment of approved fees.

Applications, including a curriculum vitae and the name and addresses of two referees, to Dr S. G. Waley, Sir William Dunn School of Pathology, South Parks Road, Oxford OX1 3RE. 1352(F)

SCHOLARSHIPS

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SCIENCE & TECHNOLOGY
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OR GEOPHYSICS 1978-1979

Normally of equivalent value to an N.E.R.C. Postgraduate Studentship plus supplementation, plus college fees and approved fieldwork expenses. Tenable for up to three years of research in a topic which falls within Shell's general field of interest. Candidates must have taken, or be taking in the year of award, either an honours degree in Geology preferably with mathematics and/or Physics at ancillary level, or an honours degree in Physics. Candidates should be not more than 27 years of age, and medically fit to work abroad.

Applications, on a special form obtainable from the Registrar, Imperial College, London SW7 2AZ, must be received by April 14, 1978. 694(H)

THE NATIONAL KIDNEY
RESEARCH FUND

invites applications for grants for renal research at units within the United Kingdom. Application forms are available from The Secretary, 184B Station Road, Harrow, Middlesex HA1 2RH (01-863 4469). 1258(H)

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UNIVERSITY OF OXFORD
INORGANIC CHEMISTRY
LABORATORY

S.R.C. C.A.S.E. AWARDS

Applications are invited from graduates (or students expecting to graduate shortly) with a good Honours Degree or an M.Sc. in Chemistry, Physics or Materials Sciences for Studentships to work on the project of which details are given below. Each Studentship will be tenable for three years, and that part of the work to be carried out in the Inorganic Chemistry Laboratory will be supervised by Dr L. A. K. Staveley.

1. Experiments designed to assist the prediction of the thermodynamic properties of liquid mixtures involving (a) the study of the equation of state of pure and liquefied gas mixtures such as methane-tetrafluoromethane to 1300 bar (b) the measurement of the excess thermodynamic functions of binary liquefied gas mixtures such as xenon-hydrogen sulphide. The processing and application of the results will be carried out in collaboration with Dr R. M. Gibbons of the Research and Development Division of the British Gas Corporation. The project therefore offers the student an excellent training in cryogenic, vacuum, high-pressure and computing techniques.

2. Calorimetric studies of zinc sulphide and zinc selenide. This will involve making heat capacity measurements on these substances from 520K down to 10K, and if necessary to 1K. The measurements which must be of the highest precision will be made on specially prepared samples which have been pretreated (e.g. annealed, irradiated) in various ways. The thermodynamic work will be supplemented by neutron diffraction studies to be carried out at A.W.R.E., Aldermaston, under the supervision of Dr K. Lewis. The aim of the co-operation is to investigate the relationship between the heat capacity of the two substances and their defect structure. The project will therefore give the student experience of precision-low-temperature calorimetry and neutron diffraction and reactor techniques.

Applications giving a brief curriculum vitae and the names and addresses of two referees, should be sent as soon as possible to Dr L. A. K. Staveley, Inorganic Chemistry Laboratory, South Parks Road, Oxford. 1330(N)

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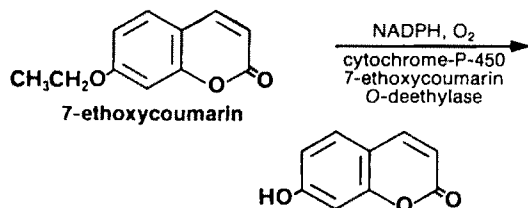


7-Ethoxycoumarin: substrate for a rapid, sensitive, fluorometric assay of microsomal monooxygenase activity

The mixed-function oxidase system in liver microsomes plays an important part in the metabolism of drugs. For this reason, sensitive and reliable tests are necessary for determining enzyme activity and consequently the drug-metabolizing capacity of the system.

7-Ethoxycoumarin has been found¹ to be an excellent substrate for the direct fluorometric determination of microsomal monooxygenase activity. The assay is based on the O-dealkylation of **7-ethoxycoumarin** to produce the highly fluorescent 7-hydroxycoumarin (umbelliferone, catalog number H2400-3).

This system involves cytochrome P-450 containing monooxygenases and is dependent on NADPH and molecular oxygen.



Since it is well known that microsomal monooxygenases are induced typically by phenobarbital and polycyclic aromatic hydrocarbons, *e.g.*, 3-methylcholanthrene (MC), the effect of these on O-deethylation of **7-ethoxycoumarin** was studied. Phenobarbital and MC induced O-deethylation of **7-ethoxycoumarin** in hepatic tissues² and in isolated rat liver cells,³ whereas only phenobarbital induced the O-deethylation in extrahepatic tissues.⁴

It has also been demonstrated that MC inducibility of 7-ethoxycoumarin O-deethylase and aryl hydrocarbon hydroxylase is determined genetically in the *Ah* locus.⁵ Poland *et al.*⁶ have shown that O-deethylase is also inducible by 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD).

An improved fluorometric assay for microsomal

monooxygenase determination via **7-ethoxycoumarin** O-deethylation has been developed recently.⁷ This *in vitro* fluorometric assay is at least *ten* times as sensitive as present methods, and has facilitated the kinetic studies of O-deethylase activity as well as a reevaluation of the use of **7-ethoxycoumarin** O-deethylation as an indicator of phenobarbital-induced monooxygenase activity in mice.⁷

This assay enables nearly quantitative recovery of the major product, 7-hydroxycoumarin, by extraction and the product is essentially free of fluorescent contaminants. Maximal fluorescence of 7-hydroxycoumarin in aqueous solution is obtained at pH 9.5 or higher. O-Deethylase activity induced by phenobarbital, MC and TCDD was studied by this method.⁷

The advantages of using **7-ethoxycoumarin** as a substrate are that the compound is not known to be carcinogenic, it is not particularly light-sensitive, and it is dealkylated to a single, highly fluorescent product.⁷ **7-Ethoxycoumarin** has been rigorously purified to eliminate as much background fluorescence as possible.

7-Ethoxycoumarin should prove to be a useful indicator of a wide range of monooxygenase inducers, particularly in cancer research.

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- 1) V. Ulrich and P. Weber, *Hoppe-Seyler's Z. Physiol. Chem.*, **353**, 1171 (1972).
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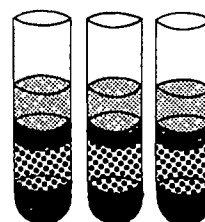


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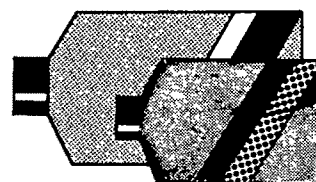
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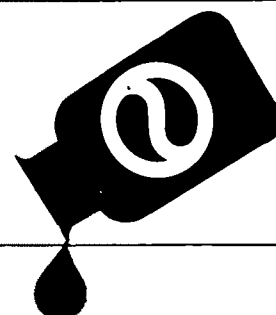
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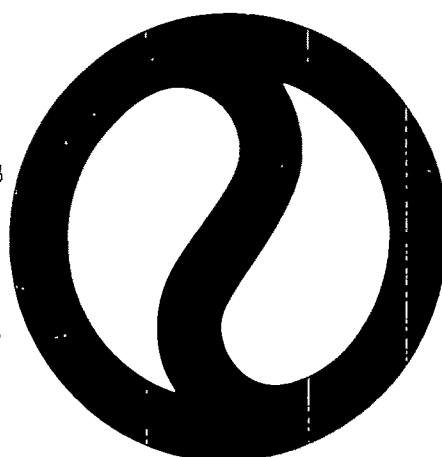


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Published weekly
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Registered as a newspaper at the
British Post Office

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Telephone: (01) 836 6633 Telex: 262024
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Binders (post-paid)

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1976 Index (post-paid) U.K. £2.50. Rest of World US\$5.00

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antenna for the detection of ultra-
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See page 641.

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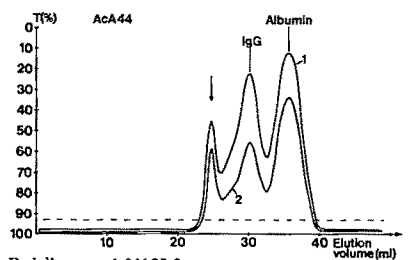
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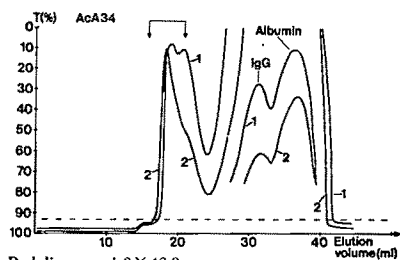
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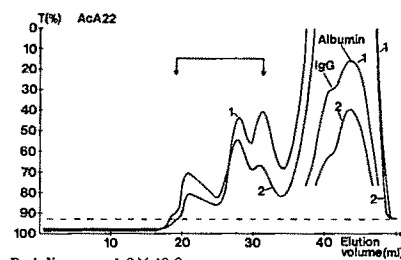
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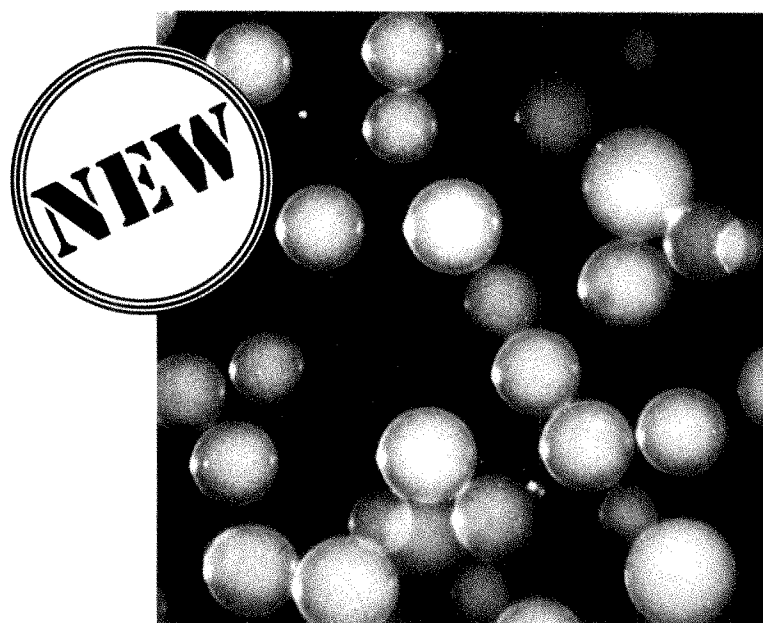
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Vietnam's dioxin problem

Much of Vietnam was sprayed with herbicides during the war. **Alastair Hay** reports on local research to assess the damage caused.

MANY times has the United States been accused of using Vietnam as a testing ground for some of the more sophisticated techniques of modern warfare. Indeed, that accusation featured prominently in much of the Vietnamese propaganda material produced during the last ten years of the war. The Vietnamese, however, were not the only accusers: United States scientists were among some of the more vocal critics of their country's military policies. Many of them made it known that they deplored such practices as the use of chemical defoliants; the implementation of weather-modification techniques; the development of plastic-pellet bombs, the intention of which was to produce shrapnel undetectable by x-rays; and the training of dolphins and porpoises to place limpet mines. All these they considered to be perversions of scientific endeavour.

Their opposition, however, had little effect upon the United States' military

activity. In many instances, information was made public only long after the event and the United States always denied emphatically that Vietnam was used as a test site for the development of military technology. But it was the first country in which defoliants were used on a large scale purely to achieve a military objective.

Originally research into 2,4-dichlorophenoxyacetic acid (2,4-D) and 2,4,5-trichlorophenoxyacetic acid (2,4,5-T) and related herbicides had been carried out during the Second World War. The British later tested their effectiveness in small-scale operations during the Malayan 'emergency' of the 1950s. But it was the success of herbicide evaluation trials in South Vietnam, Thailand, Hawaii and Puerto Rico which led to the chemicals being employed on a vast scale in Vietnam. According to a US National Academy of Sciences report of 1974 on 'Herbicides in South Vietnam', more than 10% of the inland forests, 36% of the

mangrove forests, 3% of cultivated and 5% of 'other' land was sprayed "one or more" times.

NAS estimates of the total volume of herbicide dropped on Vietnam are 17.5 million gallons, of which 11.25 million gallons was in the form of 'Agent Orange' (a 50:50 mixture of the n-butyl esters of 2,4-D and 2,4,5-T). Criticism of the use of these herbicides on ecological grounds had little impact on the actual spraying missions. With powerful advocates in the American Chemical Society defending the use of herbicides as a more 'humane' form of warfare, it required more than a concern for ecology to bring the spraying to an end.

When the US Department of Health, Education and Welfare published a report in 1969 announcing that 2,4,5-T was teratogenic in rats and mice, the herbicide programme diminished. The report was confirmed by a later independent study which established that commercial preparations of 2,4,5-T contained a highly toxic contaminant, 2,3,7,8-tetrachlorodibenzo-p-dioxin (dioxin), a teratogen at the μg per kg level in experimental animals.

Sample stocks of Agent Orange retrieved from Vietnam for analysis by the manufacturer, the Dow Chemical Co., revealed dioxin to be present in concentrations of 0.05–47 ppm. On the basis of these figures, it has been estimated that at least 100 kg of dioxin was deposited on Vietnam.

Not just a matter for science

The damage caused by herbicides is a subject of some concern to the Vietnamese authorities, and is an area of investigation for some of the country's scientists and clinicians. If the dioxin problem in Vietnam was simply one of scientific investigation, more published information would be available by now for scrutiny. But some authorities in Vietnam fear that information on the herbicide damage will be used by the military in the United States to refine its chemical warfare techniques.

Dr Tran Tri, one who voices that anxiety, is in charge of scientific relations with western countries. He says that the dioxin issue is a "complicated" matter and adds: "Of course, there is a scientific aspect to this problem which the Americans have used; our scientists, however, will not use the results in the same way". Tri wants Vietnamese scientists to do much of the general investigation on dioxin. Because of the sensitivity of the subject, he states categorically that only very few of those foreign scientists who have expressed an interest will be able to participate.

Not all Vietnam's scientists agree with Tri's interpretation. Some feel sure that the United States has more

VIETNAM occupies a long narrow strip of Indo-China. The South China Sea is the eastern boundary and a series of mountain ranges in the west act as a natural frontier with Laos and Kampuchia.

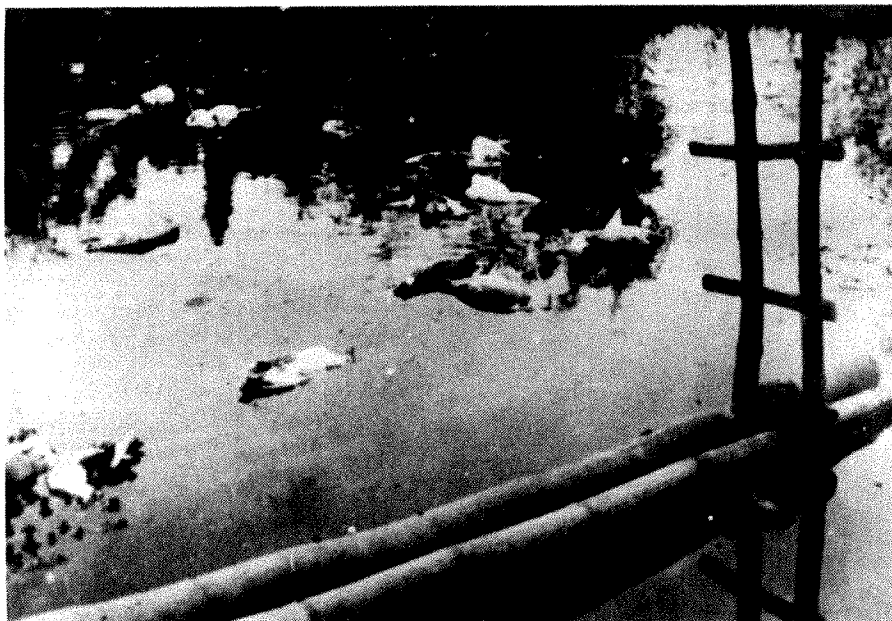
Most of Vietnam's population estimated at 50 million reside in the coastal plain and the delta regions of the Red River and the Mekong. Hanoi, the capital city, houses about 1.3 million people; Ho Chi Minh City has about 3.5 million. Five sixths of the country's population is, ethnically speaking, true Vietnamese; some 39 minority groups, of which the "Montagnards" and Chinese settlers are the largest, account for the other sixth.

Vietnam has had various occupation forces in the past two millennia. China kept her as a province from 111 BC until ousted by a national Vietnamese army in 905 AD. Subsequent attempts by successive Chinese emperors to recolonise Vietnam met with no success and the country enjoyed independence until occupied by French troops in 1858. French suzerainty in Vietnam ended in 1954, only to be partially replaced by an American presence in the south of the country. The United States, however, had a much shorter stay than France. American involvement in Vietnam finally ended with the fall of Saigon (now Ho Chi Minh City) to Communist troops in April 1975.

The war in Vietnam has exacted a heavy toll on the country with most of her resources being devoted to the war effort. The result of this is that the per capita income is exceedingly low and United Nations sources confirm that Vietnam



qualifies for urgent development aid. The distribution of aid provided by foreign donors is therefore the subject of some scrutiny. Vietnam does, however, ration food to ensure fair distribution. She has a free education programme and places great emphasis on preventive medicine. These are strategies which are generally welcomed by the international development agencies.



Vietnamese poultry killed by toxic chemicals

than enough information on herbicides. Scientists in the West working on 2,4,5-T and dioxin are of the same opinion. They point to the herbicide trials undertaken in South-East Asia in the 1960s, and to those run by the US Air Force in Florida from 1962 to 1974. Reports of the Florida experiments—which also contained information on the toxicity and biological degradation rates of dioxin—were declassified in October, 1976, to enable the Italian authorities to make use of them in dioxin decontamination work following the accident at Séveso.

Two of those most involved with the herbicide issue in Vietnam are a cytologist Dr Bach Quốc Tuyen; and Professor Ton That Tung, a surgeon and director of the Viet Duc hospital. Tuyen is in charge of the cytogenetics laboratory at the Bach Mai Hospital in Hanoi. His department was virtually destroyed when the hospital was the target of the 'Christmas bombing' of the city in December 1972.

Reporting chromosomal abnormalities

The cytogenetics department is compiling a register of the incidence of malformation in several districts both in and around Hanoi. It will be the first register to provide an accurate picture in this part of Vietnam, says Tuyen. As a scientist, he is better known for his work on chromosome mapping, and as one of the authors of two papers, published in Vietnam, which claim that the incidence of chromosome damage in people exposed to the herbicide 2,4,5-T (and its dioxin contaminant) in the American defoliation programme is higher than expected. But the number of people involved is too small to indicate statistically significant trends. This is "because we did the work

during the war", says Tuyen, and he adds: "We had no opportunity to do a controlled epidemiological survey".

Some scientists in the West have levelled another criticism at Tuyen's reports. They say that the Vietnamese have reported as abnormal a level of chromosomal abnormalities—gaps and breaks—considered normal in the West. Tuyen estimates that in Vietnam these abnormalities are of the order of 0.4%; for the West and Japan, they are much higher—at 1.0% or more. To what does Tuyen attribute this higher rate? "Chemicals. Perhaps a higher background level of radiation". And is he satisfied that his laboratory techniques are above reproach? "Yes. Scientists from Holland have verified the accuracy of our work".

Lack of contact with the West

Like many of the Vietnamese scientists, Tuyen is unable to obtain much of the published literature from the West, due mainly to the effects of war, and current lack of resources. Tuyen would welcome more contact with western scientists. He feels that it is important to conduct further studies to confirm two earlier reports by American scientists on the effects of herbicides on Vietnam.

One of the reports, in the US Congressional Record for 2-9 March 1972, states that there was a marked increase in the incidence of stillbirths in one province, Tay Ninh. The second report—by Arthur Westing to the Swedish Academy of Scientists meeting in February 1977—speaks of an increase in the rate of spina bifida and cleft palate in a Saigon (Ho Chi Minh City) children's hospital from 1966-68, the years of the heaviest spraying programmes. Tuyen is convinced that the

defoliation programme has resulted in an increase in the rate of birth abnormalities and chromosomal aberrations, but, until further information is available, he acknowledges that this conclusion is still very uncertain.

The same is true of Professor Tung's suggestion that dioxin may be responsible for an increase in primary carcinoma of the liver in Vietnam. Although Tung's publications and public statements on the subject are more categorical, he says privately that dioxin cannot be identified as the sole agent; the evidence for the link with cancer is circumstantial and there is no good epidemiological evidence to support it. He feels that there are many other possible factors to be considered, with aflatoxins and viruses obvious suspects. With a department specialising in the removal of liver tumours, however, Tung has seen an increasing number of patients referred to him for surgery, who do not come from any one particular area of the country. This makes it difficult to pinpoint any one agent.

In the opinion of many western scientists, the evidence implicating dioxin is very slim. They point out that there is a remarkably short time between the deposition of dioxin—beginning in 1962—and the appearance of liver tumours. Most known carcinogens have a latency period of between 20 and 30 years.

The final group of scientists involved in the assessment of herbicide damage are the zoologists at the Vietnam Scientific Research Centre in Hanoi. Dr Ngoc Quang, one member of this group, has some preliminary evidence of changes in the pattern of bird life in sprayed areas. "Normally", he says, "there is a population of about 40 peacocks per hectare. In areas sprayed with herbicides, there are none". He also reports a reduction in the pheasant population, and adds that scientists in Vietnam are "anxious to have help to assess the damage to our fauna. The changes we have recorded may not be due to the herbicides; there may be other causes."

Resolving the 'dioxin problem' is not one of Vietnam's top priorities; there are too many other areas, medical and agricultural, requiring urgent, immediate attention. What the long-term effects will be it is impossible to say; the evidence is just not available. With contamination on this scale, Vietnam is in a unique situation, not to be envied. It is to be hoped that, when she asks for assistance from the scientific world, it will be promptly forthcoming. □

Alastair Hay was recently in Vietnam as a Nature travelling fellow. He was supported in part by the London Technical Group.

The great American dream machine runs out of fuel

David Dickson discusses the dilemmas facing the Carter administration over the future shape of the US space effort

FIRST the good news. President Carter's administration has proposed that the space science budget of the National Aeronautics and Space Administration (NASA) be increased by 26.7% to a record level of \$513 million—out of a total NASA budget of \$3,305 million—in 1979.

Now the bad. There are no plans to start any new planetary missions in 1979, and it now appears that apart from Project Galileo, the Jupiter Orbiter/Probe given the go-ahead by Congress last year, no major new planetary programme is likely to be under way until the mid-1980s.

It is almost exactly 20 years since the US entered the space race with the launch of the Explorer satellite from Cape Canaveral on 30 January, 1958 as a hastily arranged response to the trauma of being beaten into space by the Russian Sputnik. It is also the 20th anniversary of the National Aeronautics and Space Act of 1958, which established NASA. And last week, in celebration of the latter event, the Senate Committee on Commerce, Science and Transportation held a symposium on the future of space science and space technology.

If the future facing space science 20 years ago lay full of hope and promise, the symposium indicated how confused and uncertain the picture is today. This confusion is reflected in doubts about the future directions that NASA should pursue, indeed doubts about the whole shape of the US space effort, including space science.

Two areas are doing well. The first is space astronomy, with exciting results on X-ray and gamma-ray emission already coming from the High Energy Astronomical Observatory (HEAO), launched last year, and more promised from the Large Space Telescope, which is being started in the 1978 budget. The second area which is also expanding, and which reflects the administration's desire to see an emphasis placed on the basic research necessary to support applied research, lies in the area of space applications. Here the relative complexity of some new applications of satellite technology, such as earth resources reconnaissance and climatology, at least in comparison to earlier applications such as telecommunications, have given rise to a significant increase in the supporting science.

But there are two areas in which current frustrations are running high, and short-term expectations—in spite of vigorous pressure—remain low. The first is in what has been called "space industrialisation", where the imagination of space engineers, with plans for mining asteroids for important minerals and creating vast solar receptors to beam energy down to earth, have so far not been matched by any major commitment from NASA. The agency claims that it is holding back work on large space structures until it sees the outcome of the Space Shuttle programme.

Planetary science

Planetary science is the second area in which NASA appears to be holding back from any major future commitment. Admittedly the present situation for planetary science is not too bad, largely because of the success of scientists in getting the Jupiter Orbiter/Probe into the 1978 budget. (Indeed, the main reason for the substantial 26.7% increase in the 1979 space science budget is last year's acceptance by Congress of this and the space telescope, both projects requiring substantially more funding in the second rather than the first year).

But the Jupiter project only survived in NASA's programme after a considerable battle in Congress, which required mustering the full letter-writing potential of the planetary science community. Although accepted by the Senate, the project was rejected in a surprise move by the House Appropriations Committee under the chairmanship of Mr Edward Boland (Dem-Mass), and only scraped through following compromises made at the conference stage.

Less fortunate has been a proposed project to send a spacecraft into a polar orbit around the Moon, making use of a set of matched sensors that would have provided a survey of the whole planet from a height of 100 km. This had been proposed within NASA in three successive years, and twice (for 1978 and 1979) put forward by NASA to the Office of Management and Budget, only to be rejected on each occasion. It now seems unlikely that NASA will be prepared to put forward this project again, preferring to rethink how it should reapproach the

Moon at a later date, possibly in five or ten years' time.

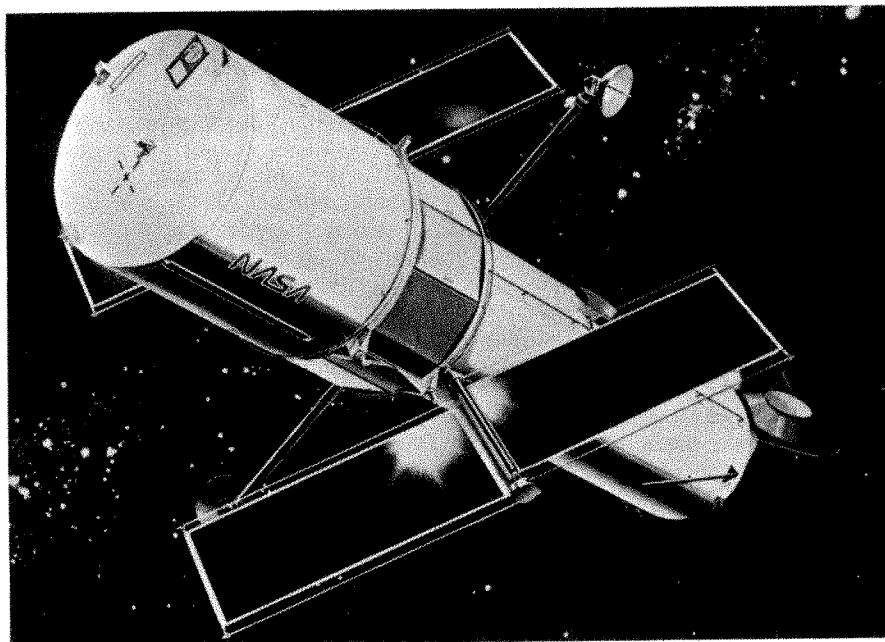
Two other projects discussed within NASA failed to get the agency's support. The first was the proposal to send a spacecraft to rendezvous with Halley's Comet, due to make its once-in-75-years approach to the sun in 1986. A second project was a proposed follow-up to the Viking mission to Mars, either sending a roving vehicle to the planet to gather more information about its surface, or to arrange for a sample of Mars rock to be brought back to earth for analysis.

The possibility of a Mars project is still receiving enthusiastic backing from scientists, particularly those interested in investigating the distribution of organic matter in the universe. And there will be a considerable push this year to get a trip to Mars in 1986—the next date when the planet will be in a suitable position relative to the Earth—into the 1980 budget.

However, it will have to compete with another equally attractive proposal, that to send an imaging radar device to orbit Venus in order to make the first accurate measurements of the planet's surface through its dense clouds. A Venus project could be particularly attractive for two reasons. Firstly, there are already two planned Pioneer trips to Venus planned for launching in May and August of this year, and interest in the planet is therefore likely to be high. Secondly, and perhaps politically more important, is the fact that the Russians are planning both a fly-by mission, probably with an orbiter, and also a joint mission with the French that will involve dropping a balloon into the planet's atmosphere.

Apart from these there are a number of additional projects being proposed, each with its group of supporters. One is to send a mission to Titan, the largest of Saturn's moons, whose atmospheric conditions make it of particular interest to space scientists with biological interests. Those disappointed by the failure to gain support for a mission to Halley's Comet still contend that a comet mission of some kind would provide invaluable information about the origins of the universe. And there is also a project being discussed to reintroduce manned investigation in the relatively near future with a mission to one of the many small bodies, such as the nuclei of burnt-out comets, that orbit the Sun close to the Earth.

Each of these projects would be fruitful from both a scientific and a technological point of view. The main problem facing planetary scientists, however, is how to secure that extra bit of commitment from the administration necessary to obtain the vast amount of resources that each would require.



Artists' impression of NASA's space telescope

Victims of earlier success

The size of the projects, the uncertainties involved, and the long-term nature of any pay-off, make it impossible to apply with any degree of accuracy the type of cost-benefit analysis that is increasingly being required of R & D efforts. As Dr Noel Hinners, associate administrator for space science at NASA, points out, "scientists have desires, not requirements."

But in their failure so far to gain the necessary backing, the hawks of both space technology and space science are partially the victims of their own earlier success. On the one hand, the apparent ease with which the space bonanzas of the 1960s were achieved, and the technological euphoria which they generated, has obscured the type of commitment needed to secure success; recent launcher failures and technical problems with Space Shuttle engines have made both administrators and politicians more cautious in their projections of feasibility.

The second spin-off from earlier successes has been that, by putting the emphasis on the more competitive aspects of getting places first, it has been made comparably harder to argue for follow-up missions of equal, if not greater, scientific value. Both the proposed lunar and Mars projects which failed to receive funding for 1979 suffered partially from this problem.

The resulting situation was expressed at the Senate symposium by Professor James Arnold, of the Department of Chemistry at California University, La Jolla, and a leading advocate of the lunar mission. "The lunar and planetary science community currently feels a great frustration based on the paradox that we have very exciting things that we know how to do, but

budgetary restrictions on doing them," he said.

This frustration, shared with those who would like to see NASA advancing rapidly into large technological structures, finds a frequent echo in Congress from those who argue the need for the US to maintain a position of superiority both in leading the pursuit of new knowledge, and in maintaining a technological advantage over other nations (NASA's formal role is according to the National Space Act to preserve the role of the US "as a leader in aeronautical and space science and technology").

But this style of political debate, however attractive it may have appeared during the Cold War period when politicians could play on the fear of Soviet technological superiority, is out of place in the current administration. The form of hegemony that President Carter sells is moral, rather than technological (just as the success of *Star Wars* is based less, as the space hawks like to suggest, on the inherent attractions of space travel as on the apparent victory of morality over technology).

Thus, however much he is attacked for showing a lack of imagination, space bonanzas, whether scientific or technological, are not Carter's style; like other candidates for federal aid, they require pragmatic justification, in the way that NASA is putting increasing emphasis on the "useful applications" of space technology—and the science necessary to achieve it—rather than the more glamorous aspects.

Dr Robert A. Frosch, NASA's newly appointed director, is under pressure from both Congress and the aerospace industry to take a more "imaginative" approach. But he is proceeding cau-

tiously, and as a result finds himself "in the somewhat paradoxical situation that as steward of NASA I have been cast in the role of the conservative, a brand new position for both me and the agency."

Looking to the future

As the NASA budget for 1979 comes under scrutiny from Congress, there are likely to be several attempts to increase both its size and its scope. Already Representative Olin Teague, for example, has been persuaded to table a resolution pressing the investigation of space resources. And representative Ronnie G. Flippo of Alabama echoed a widely-held feeling when he told the House two weeks ago that the 1979 budget was in general "a *status quo* budget, lacking in the kind of action, boldness and innovation which should characterise scientific leadership."

But in general space is much lower on Congress' priority list than it was ten years ago. Pay-offs must not only be promised, but their feasibility demonstrated. The Space Shuttle, for example, succeeded in getting through Congress largely as a result of the economies that could be demonstrated for a re-usable space vehicle, and its potential commercial viability.

The changing environment is further reflected in the uncertainty over the future of NASA, itself partially a victim of its own success. Some claim that the agency should now shift its concern from the means to the goals of space activity, helping to build what one enthusiast calls "the new space America."

Others feel that NASA's responsibility should remain essentially exploratory and experimental, although most space scientists seem to agree that the agency should remain responsible for both the basic sciences and the investigation of its implications, since the former will only thrive if conducted in a symbiotic relationship with the latter.

At present the administration in general—and President Carter in particular—seem uncertain about the direction in which NASA should turn. A study of the national space effort conducted as a result of a presidential memorandum last year by Dr Norman Brown, Secretary of State for Defense, is said to have disappointed the administration in its conclusions.

More significant for space science will be a "public declaration" on the future shape of civil space activity, including NASA, which is currently being prepared by Dr Frank Press's Office of Science and Technology Policy. Some form of statement from OSTP is expected next month; but a renewed commitment to major programmes in space still seems a long way off. □

Fermilab director resigns over funding

In an effort to stabilise the funding of high energy physics (HEP), the US Department of Energy has decided to recommend that the HEP budget should be kept at a constant value of \$300 million (in 1979 prices) over the next few years.

According to the department, this sum—which breaks down into \$200 million for operating costs, \$40 million for equipment, and \$60 million for construction—will guarantee an active HEP programme based on three national centres, the Brookhaven National Laboratory on Long Island, the Fermi National Accelerator Laboratory in Illinois, and the Stanford Linear Accelerator Center (SLAC) in California.

However, although the department's proposed commitment is considerably higher than two years ago, when the HEP budget dropped to about \$240 million after reaching a peak of about \$500 million (both in 1979 prices) in 1970, it is still less than many scientists would like. And in protest at the department's decision not to speed up the construction of the energy doubler at Fermilab, Dr Robert Wilson, director of the laboratory, has carried out a threat made two months ago and submitted his resignation.

The Department of Energy's decision to keep HEP funding constant at a level slightly higher than the \$290 million proposed for fiscal year 1979 was taken after long and detailed discussion with the high energy physics community, in particular over the construction of the 400 GeV proton-proton colliding beam facility (ISABELLE) at Brookhaven which the department has proposed should start in 1979.

"Before agreeing to support ISABELLE, I asked what would make sense in terms of a long-term high energy physics programme?", Dr John Deutch, director of the department's Office of Energy Research, said last week. "I was not prepared to support the construction of a major new facility unless it was in the context of a balanced and aggressive high energy physics programme." Dr Deutch said that the decision to maintain a constant budget in real terms over the next few years was consistent with the consensus of the high energy physics community over the type of support that was required, and that it would provide some stability for future planning. A similar scheme was also being developed for funding nuclear physics.

The decision was welcomed by Dr Sidney Drell, deputy director and chairman of the department's High Energy Physics Advisory Panel (HEPAP), who pointed out that ever since the Ramsey report of the early

1960s, physicists have been asking for a stable basis for long-range planning. "Stable funding expectations will be very useful to us in terms of allocating resources, particularly in view of the fact that major HEP instruments can take seven years or so to build", he said. He added that the general level of funding was only "the minimum viable level" compatible with a three-centre programme, and there would still be many opportunities lost for good physics. According to Dr Drell, the proposed funding levels represent a 25% drop in operating funds for HEP facilities from their peak at the end of the 1960s.

Dr Wilson's decision to resign as director of Fermilab is a result of his failure to persuade the Department of Energy to bring forward the construction of the energy doubler, which by adding a second ring of superconducting magnets will eventually enable Fermilab both to save power costs and to reach energies of 1,000 GeV or more. Although the department is proposing to allocate \$10 million towards construction of the doubler in the fiscal year 1979, Dr Wilson had asked for

\$35 million, which would have been sufficient to complete the project during the year.

In his letter of resignation to the Universities Research Association, which runs Fermilab, Dr Wilson said that current restrictions of funding meant that the laboratory was operating at about half its potential capacity, a predicament that was particularly serious in view of the relatively high funding enjoyed by the European Centre for Nuclear Research (CERN) in Geneva, which recently brought its own 400 GeV accelerator into successful operation.

"Our scheme to leapfrog CERN's financial advantage by increasing the Fermilab proton energy to 1,000 GeV through the application of superconductivity has been confounded by indecisive and subminimal support," Dr Wilson said. He added that he had felt he was unable to continue to give the impression that he could responsibly direct Fermilab without a substantial increase in funding.

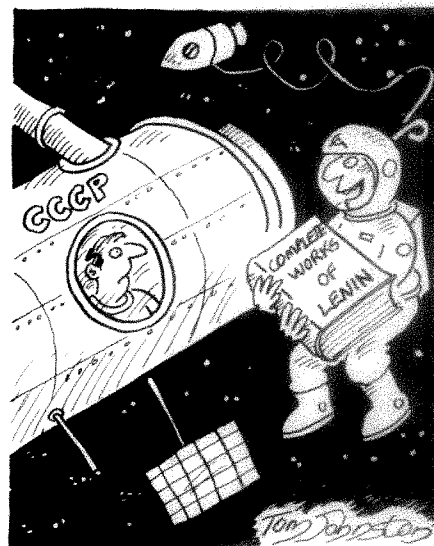
Although Dr Wilson is resigning as director, he has said that he intends to remain at Fermilab to work on the energy doubler project.

David Dickson

Salyut-6 used for weightlessness experiments

THE long sojourn of cosmonauts Yuri Romanenko and Georgii Grechko aboard Salyut-6 represents far more than an attempt to recapture the space endurance record. Soviet space planning has always been based on the idea of permanent orbital stations, indeed, Tsiolkovskii, "the father of Soviet cosmonautics" envisaged such stations as a *sine qua non* to manned lunar or interplanetary flight. The rumours which followed the Soyuz-11 disaster, which attributed the cosmonaut's deaths to the effect of prolonged weightlessness, were traumatic to space planners and amateur enthusiasts alike, and although subsequent investigations proved that the tragedy was in fact caused by a defective seal and consequent depressurisation, research into the physiology of weightlessness is a major concern to the Soviet manned space programme.

Unfortunately, weightlessness research is something which must largely be carried out *in situ*, and there is little scope for ground-level simulation. A 'hydro-weightlessness' tank is used to train cosmonauts for the difficult mechanical operations of station maintenance. Poland, as part of its contribution to the *Interkosmos* project, is said to be working on the biomedical effects of weightlessness, and an interesting experiment was recently



'You did say any kind of moral support, comrade!'

completed in Leningrad where for six months 18 volunteers lay "in a very inconvenient posture, with their feet several degrees higher than their heads", able to wash, shave, eat, and read, but "only allowed to turn from side to side, without rising even a little".

That experiment, which was presumably intended to simulate space-flight demineralisation, proved, according to its scientific director, Professor Leonid Kakurin, to be a great success.

Once the volunteers resumed ordinary life, their physiology quickly returned to normal, so that, according to Kakurin "space flight is not limited to six months. The human organism has genuinely unlimited possibilities".

On board experiments, however, are the only sure way of determining the effects of weightlessness. The biological programme of Salyut-6 includes both human and non-human tests. Certain of the latter seem somewhat esoteric—as, for example the result that tadpoles hatched in space swim in spirals, while earth-hatched tadpoles swim in a more "disorderly" manner. Other Salyut-6 experiments, monitoring the total effect of space rather than weightlessness alone, include the Franco-Soviet Cytos experiments on micro-organism cell division, a genetic investigation using *Drosophila* (banned from Soviet research at the height of Lysenkoism) and the somewhat ominously named "Medusa" experiment which compares the effect of space-flight on biopolymers in and outside the station.

The main source of medical data is, however, the on-board monitoring of cosmonauts. The Salyut-6 tests have included electrocardiograms, encephalograms and rheograph and manometer investigations, used at "rest" and after exercise on the "running track" and "veloenergometer". A special self-contained microanalysis kit was used to enable the cosmonauts to take samples of each other's blood; these were returned to earth by the Soyuz-26 craft.

The possibility of returning specimens in this way has greatly extended the facilities for maintaining the cosmonauts' health and well-being. Similarly the use of unmanned supply rockets will enable any necessary medicines to be taken into orbit. Progress-1 did, in fact, carry a replacement medicine chest—since although the original one had not been breached, it was felt that certain medicaments might have grown stale and lost their efficacy.

The use of replacement crews and supply rockets, however, has far more significance to cosmonaut health than simply that of keeping the life-support systems operative and the supplies recharged. The Salyut-6 planners have laid great emphasis on the physical comfort and psychological well-being of the cosmonauts. Station noise has been reduced by siting the motors "further astern" and installing "quieter switches". A shower has been installed (a triumph of ingenious design) and semi-rigid spacesuits are used for EVA which are said to simulate gravity by directing pressure to the lower parts of the body. The on-board menu includes over 60 named brands of food.

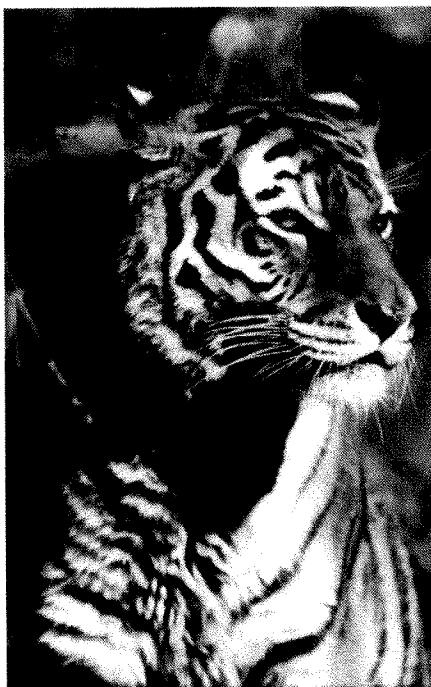
Nevertheless, all is not entirely

happy aboard Salyut-6. The TV set ferried up by Progress-1 was said to be urgently needed, to keep the crew in touch with world events. One cosmonaut mentioned that after 45 days in orbit there was a need for "any kind of moral support"; it was a great boost, he said, to know that the completed experiments returned for processing via Salyut-26 had turned out well. Clearly, one of the main problems of small-crew long-term space-stations is

likely to be that of psychological isolation—a factor which will undoubtedly increase as such stations become a matter of routine rather than headline news. One can only hope that in the future, relieving or supply crews will be less careless than Dzhaniybekov and Makarov, who in the excitement of undocking from the first four-man Salyut-link-up left behind the letters home of Romanenko and Grechko.

Vera Rich

USSR moves to protect rare animals



Vanishing species: the Amur tiger . . .



and goral

THE Soviet Union is preparing new legislation governing the conservation of rare fauna and restrictions on hunting. The December 1972 Resolution of the Central Committee and Supreme Soviet "on increasing the protection of nature and improving the use of natural resources", placed an emphasis on ecology that had been largely lacking in Soviet official thought, but so far its provisions have proved impossible to implement.

According to N. Eliseev, head of *Glavokhota* (Principal Hunting Trust) of the Russian Soviet Federative Socialist Republic (RSFSR), the poaching of rare and protected species is still rife. The RSFSR is by far the largest of the Union Republics, comprising Russia proper and the major part of Soviet Asia. Eliseev's department is thus responsible for 80% of all fur-bearing animals and 90% of all bird-life in the Soviet Union, including such rare and vanishing species as the Amur

tiger, the goral and the desman (source of musquash).

Hunting is still an important industry (since in much of the Soviet Union fur is a necessary rather than a luxury article) and it is governed by a somewhat complicated system of inspections and licences. But the poachers often manage to circumvent the various controls by making rapid safaris in fast cars and motor boats. Poaching, or at any rate, breaches of the hunting regulations on the part of licensed hunters, are common.

The new laws, says Eliseev, "will intensify the protection of wild animals, birds, fish and other fauna". Presumably this means (though he does not say so) severe penalties for poachers and dishonest hunters. Eliseev, however, stresses the positive approach to conservation, demanding a fundamental change in social attitude: "An end must be put to the consumer attitude towards nature". □

Germany's energy plans

THE West German government presented its latest programme to safeguard Germany's future energy supplies last December. An important feature of the programme concerns the efficient use of energy, especially for use in private households which accounts for the largest share of all Germany's energy consumption.

As heating makes up for 80% of private consumption, in future all new buildings will be legally required to conform to building regulations aimed at improving insulation. The government is also encouraging private owners to improve the insulation of existing buildings by offering them grants of 25% of the initial cost of improvement up to DM12,000 per purchase (£1=4DM) over the next five years. The grant also applies to the installation of solar collectors.

The new energy programme is clearly in favour of nuclear energy. Without it, the government feels that it could not guarantee an electricity supply in the long term. But just how much nuclear power station capacity is necessary is a matter of dispute particularly amongst the governing parties.

In future, however, new nuclear power stations will be sanctioned only if the problems of radioactive waste

disposal are solved. And the German view is that this can only be done by building a central plant to reprocess the spent fuel elements and to take care of the final disposal of radioactive wastes.

A site for such a plant has been chosen at Gorleben but the local population are protesting about its construction. So at the moment additional storage ponds are being built to store spent fuel elements from the power stations. The basic commitment to nuclear energy of both the federal government and the parties in the Bundestag will, therefore, only be realised if the radioactive waste disposal problem is solved.

However, the German government is also looking to coal for future energy needs. But German pit coal is very expensive and the present production of 90 million tonnes per year can be maintained only with considerable state subvention. Government and industrial research into non-nuclear energy sources is also being strengthened, the conversion of coal into other products being central to the government's programme. Research on alternative energy sources, such as solar and wind energy, is also receiving government support.

Werner Gries

World Bank's new health commitment

HAVING for years fought shy of involving itself in activities concerned with health, the World Bank has now taken a second step in the opposite direction—the first being its co-sponsorship of WHO/UNDP Onchocerciasis Control Programme (OCP) in the Volat River Basin. The new commitment was confirmed at a meeting at the beginning of February at WHO Headquarters in Geneva, with a 'memorandum of understanding' between the same three partners as co-sponsors of the Special Programme for Training and Research in Tropical Diseases. In general, the arrangement follows the same lines as that for the OCP, the Bank being responsible for administering the Tropical Diseases Research Fund, contributed by governments and organisations for projects to be executed by WHO and UNDP.

As expected, the cooperating parties' interests will be coordinated by a Joint Consultative Board. The board is expected to hold its first meeting in November this year. Its 30 members will include 12 representatives of governments, selected by the contributors; 12 selected by WHO's Regional Committee from among those countries directly affected by the diseases, or supporting the fund with scientific and technical assistance; three from among cooperating parties not otherwise represented; and one each from three sponsors of the programme. The latter will also have their own standing committee, meeting twice a year, concerned with technical and financial oversight of the programme.

As in the case of the OCP, there will be a scientific and technical advisory committee, concerned not only with advice on these aspects of the programme, but also with continuous independent evaluation of all programme activities. The 15 to 18 members of this committee will serve in their personal capacities, covering the whole range of biomedical and other disciplines with which the programme is concerned. The initial impetus will be maintained by interim meetings of the various groups before their formal establishment in November. A programme coordinator at the headquarters of the WHO will be responsible for the management of the programme.

As to financing, the meeting ended with some \$11 million pledged by various donors for the current year's activities. Moreover several large donors indicated that they would be making longer term pledges to cover at least the next five years of the programme.

Peter Collins

Sighting the Yeti's relatives

FOLLOWING reports of a possible "Loch Ness" monster in Lake Kos Kol, Soviet scholars have postulated a possible "relation" of the Yeti. The Institute of Language, Literature, and History of the Yakut Branch of the Academy of Sciences of the USSR has collected numerous accounts of sightings of a creature known locally as the "Chuchunaa", a name which apparently is connected with the Yakut word for "fugitive" or "outcast".

According to informants, the Chuchunaa is over 2m tall, uttering deerskin, and is unable to talk, uttering only a piercing whistle. He is described as a meat-eater, and is said to have the habit of creeping up to settlements and stealing food. When the Chuchunaa sights a hunter or reindeer-herder, he usually takes flight, but on occasion, it is said, will pick a fight. (No data have been released as to the outcome of such encounters.)

The best sightings of the Chuchunaa seem to occur in summer at dawn or late dusk. Hunters, mushroom pickers and the like who are out at this time have reported seeing a wild man, with arms hanging below his knees, barefoot and dressed in skins. The face is said to be large, human-like but very

dark, with a protruding brow "like a peak", long matted hair, and a full beard—larger than that of a man. At such times, the Chuchunaa has been reported to be feeding on berries, tearing them from the bushes with both hands.

According to Semen Nikolaev, a senior staff member of the Yakut Branch of the Academy, "almost all witnesses speak of the Chuchunaa as a reality without the fantastic detail so characteristic of legends". (To a Westerner, the above description seems very much the stuff of which legends are made—and one cannot help wondering whatever Nikolaev would classify as "fantastic"—Baba Yaga riding on her pestle, maybe?). Since the details of many sightings coincide, Nikolaev and his colleagues seem willing to admit the postulate that the Chuchunaa represents the last surviving remnant of the "Palaeoasiatic aborigines" of Siberia, who have retreated to a last refuge in the upper reaches of the Yana and Indigirka rivers. Indeed, since the last reliable sighting dates from the 1950s, some of the more pessimistic experts think that the Chuchunaa may have died out during the last two decades.

□

Chinese exchanges easier

CHINA has begun trying to achieve the four modernisations—agriculture, industry and defence with science and technology as the 'key'—in accordance with the policy laid out in Chairman Hua's Political Report to the Party Congress last August. The plan for agricultural modernisation over the next three years was revealed on 26 January in a report to the third national conference on agricultural mechanisation.

In spite of these moves towards achieving China's new goal, however, discussions are still taking place on the future of many aspects of national life. In education, for example, it has been reported that the advice of a top leader has been sought on a question not resolved at ministry level. It is unlikely, however, that this concerned the egalitarian drives to set up an 'open university' via the national television network and to make universal by 1985 middle-school education in the cities and junior middle-school education in rural areas. The

overall picture, however, is that progress in education is regaining momentum; an All-National Conference on Education will be held after the one on science in

China is also implementing plans to increase international academic exchange. The situation report delivered last December by Fang-Yi, Vice-President of Academia Sinica, reiterated that China is striving to learn advanced science and technology from foreign countries whilst keeping to the principle of self-reliance and independence. Already, some scientists of Chinese origin have been led to believe that they will be invited to China at some stage in the near future for short term teaching and/or research. And there is also no question that China will employ many foreign experts.

An ordinary tour of China can be simply arranged. Last month's issue of *China Reconstructs* published a guide for prospective applicants, either single or in a group. A technical visit, such as a visiting professorship, will probably

have to go through personal connections or organisations such as the revitalised Chinese Scientific and Technical Association, whose acting chairman is Chou Pei-yuan of Peking University. Chou (a physicist) may in fact be regarded as in charge of academic matters in research and education, and Fang Yi as in charge of political and general concerns.

The Japanese, however, have gone some way to taking the initiative in fostering exchange with China: an Association for Japanese-Chinese Scientific Exchange was founded in Tokyo last December. The French also recently signed a Science and Technology Agreement with China covering, amongst other cooperative schemes, the exchange of scientists. Incidentally, M Barre's state visit to China also resulted in another development: Hua's acceptance of an invitation from Giscard d'Estaing to his country. That will make France, an old friend of China's, the second country in the world that a Chinese Communist Party Chairman has ever visited.

T. B. Tang

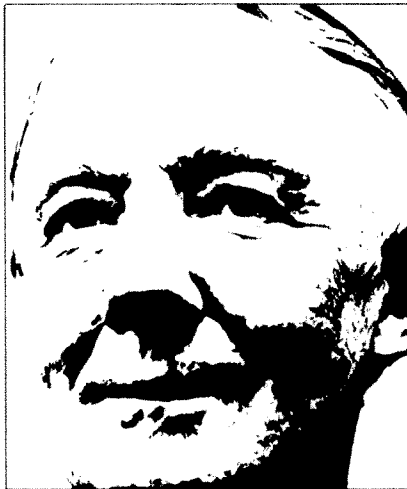
Most countries have their own units of length, weight and volume, and several systems for measuring temperature have been used in different parts of the world. These different units present difficulties to travellers, and to the communication of scientific results between nations. Many years ago, on a walking holiday in Sweden, I set off for a village which a signpost said was three miles away. After striding along at a reasonable pace for nearly two hours I saw a second sign saying that I still had to cover two miles. Thus I learned that one Swedish mile was equal to six English miles.

On another occasion at a seaside resort in Denmark I saw a notice which said that the temperature of the sea was 16 degrees. I hesitated to bathe, for this seemed a little chilly, but when I overcame my reluctance I found the water warmer than expected. The temperature had been recorded on the Réaumur scale, and corresponded to 20 degrees Celsius, or 68 degrees Fahrenheit.

It seems sensible to come to some agreement so that the same units are used internationally. For a long time scientists in Britain and many other countries have followed two systems, metric in the laboratory, and 'traditional' outside. We are now trying to change and are using the metric system everywhere. Unfortunately, this rational change makes life less interesting. It was always pleasant to find maunds and seers in India, and quite easy to make the necessary conversions. I was glad to find recently in Mauritius that land

is still measured by the arpent (1.043 acre). British farmers are urged to go metric, but they still think, and usually talk, of yields in hundredweights per acre, though the bushel has largely disappeared.

Uniform units



KENNETH MELLANBY

Those of us brought up in the laboratory on what are now old-fashioned metric units find the SI system difficult to use. I really cannot understand why I must stop talking about cubic centimetres, for this is a sensible unit and is easily understood. The change to millilitres seems to be unjustified.

Even more important than using the same units (for these can easily be con-

verted) is to have the same meaning for words. In Britain and most of Europe the billion has always been 10^{12} . North Americans, thought by Europeans to be prone to exaggeration, give the word the value of only 10^9 . I despair of converting Americans back to the correct use of the term, and see no reason to follow their bad example. I therefore believe that the term has become so ambiguous that it should no longer be used. As an editor I ban it from my journal. Recently I have been shocked to see that *Nature* has allowed the word, with its American use, to appear in its columns. I hope that all my readers will support my campaign for the total abolition of the billion in any form.

In Britain we have even decimalised our currency, and lost our useful shillings and pence. One more exotic coin, the guinea, has not yet disappeared entirely. It is still used for selling expensive race horses and to pay for private medical treatment. The guinea was originally the same as the pound sterling, and was made up of twenty shillings. However, from 1717 gold sovereigns (pounds) made of Guinea gold from West Africa were found to be so pure that they were officially valued at twenty-one shillings. I recently received a bill for dental treatment for fifty guineas; this was converted by a young clerk into £55. She apparently thought that with decimalisation the guinea was now £1.10 instead of the original £1.05. No wonder people think that decimalisation has helped to increase the rate of inflation.

correspondence

USSR bars Israeli scientist

SIR,—We wish to protest against the discriminatory practices of the Soviet Union towards Israeli scientists wishing to participate in international scientific meetings held in the USSR.

The Fourth International Meeting on Ferroelectricity (IMF-4) was held in Leningrad on 18-23 September 1977. The meeting was organised by the Academy of Sciences of the USSR under the sponsorship of the International Union of Pure and Applied Physics, the International Atomic Energy Agency, and the European Physical Society. We had both delivered papers at the Third International Meeting on Ferroelectricity in Edinburgh in 1973, and we were looking forward to participating in IMF-4. However, at every stage—soliciting information regarding the meeting, inquiring as to the status of our submitted manuscripts, attempting to obtain an entry visa to the USSR—we, and several other Israeli colleagues, encountered incredible obstacles, which ultimately led to our being unable to attend the meeting. Our individual experiences were as follows:

Sidney B. Lang submitted the abstract of a paper on 29 March 1977 to Professor G. Smolensky, Chairman of IMF-4 in Leningrad. Receipt of the abstract was never acknowledged, nor was information sent regarding the status of the paper. After seeking the assistance of Professors W. Cochran (University of Edinburgh) and W. J. Merz (R.C.A., Zurich), one of us (S.H.) received a letter from Professor Cochran, dated 30 June 1977, stating that he had received a cable from Professor Smolensky that the papers of Lang and Havlin had been accepted. Because of the impossibility of an Israeli receiving a visa to the USSR without first travelling to Western Europe, and lacking any official documentation from the Organising Committee that could be used in requesting a visa from Soviet embassies in Europe, Lang was forced to withdraw his paper on 25 August 1977. On 16 September 1977, two days before the meeting opened, Lang received the 'second circular' about the meeting, mailed from Romania on 30 August. This circular bore no date, but it specified a number of deadlines between 30 April and 18 June.

Shlomo Havlin submitted the abstract of a paper on 7 February to Pro-

fessor Smolensky and as in the case of Lang, heard nothing. On 8 July the Secretary General of IUPAP, in response to requests for assistance by the Israel Physical Society, cabled that Havlin's paper was included in the meeting programme, and that he need only apply to Intourist for an entry visa. On 9 August, Havlin arrived in Paris and immediately applied for a visa at the Intourist office. At every subsequent visit to the office as well as to the Soviet Embassy, he was assured that his visa would be issued "tomorrow". The seemingly sincere promise that the visa would surely be forthcoming led Havlin to remain in Paris beyond 8 September when he had planned leaving for Leningrad. On 11 September, still with no visa, Havlin realised there was no point in continuing his battle and left Paris.

Although the organisers of the Leningrad meeting had made firm promises to IUPAP, Professor Cochran, and Professor Merz, the Soviet authorities refused to issue a visa. From our experiences, it is obvious that Israeli attendance at this international meeting was prevented by the Soviet Union.

We suggest the following to our fellow physicists and to the officials of physics organisations:

- International organisations should not sponsor meetings in the USSR without firm guarantees from the Soviet government that these meetings will be open to citizens of all nations without exception. Promises from Soviet organising committees should not be accepted *in lieu* of official Soviet government commitments. Scientists should write to the officers of the physics organisations to which they belong, demanding that this principle be strictly adhered to.
- Scientists should refuse to attend meetings in the USSR unless they are certain that the Soviet government has, in fact, guaranteed that the meetings will be open to citizens of all nations without exception.
- Letters should be written to the Soviet organisers of IMF-4 protesting the discrimination that was practised towards Israeli scientists. The organisers were:

Chairman:

Professor G. Smolensky
A.F. Ioffe Physico-Technical Institute
Academy of Sciences of the USSR
Leningrad, 194021
USSR

Vice-Chairmen:

Professors L. Shuvalov and I. Zheludev
Institute of Crystallography
Academy of Sciences of the USSR
Moscow, 117333
USSR

We believe that the holding of meetings without firm adherence to the above principles is totally incompatible with the international character of science and scientific research.

SIDNEY B. LANG

*Ben-Gurion University of the Negev,
Beersheva, Israel*

SHLOMO HAVLIN

*Bar-Ilan University,
Ramat-Gan, Israel*

Burying high-level wastes

SIR,—The disposal of intermediate and low level radioactive wastes in salt deposits of Permian (about 220 Myr) age is proceeding and proposals have been made to treat high level wastes in a similar fashion. When evaluating the suitability of these formations for this purpose, the proponents stress the antiquity, aseismicity and availability and conclude that they are "extremely stable" (*The Management of Radioactive Wastes*, IAEA, Vienna; 1977). Most geologists, however, would consider that rocks of this type are very unstable. Widespread halokinesis is well-documented and the role of diapirism in the formation of stratigraphic traps favourable for oil and gas accumulation is particularly significant.

In rocks containing potassium the radioactive decay of ^{40}K forms radiogenic ^{40}Ar , resulting in a high relative abundance of this isotope in the atmosphere. In early experiments designed to evaluate the possibility of using this decay scheme to measure geological time the ^{40}Ar concentrations in samples of evaporite were measured. In almost all cases it was found that significant argon loss had occurred. This was attributed to the instability of these strata and their susceptibility to dissolution by fluids (such as percolating ground water) and subsequent recrystallisation which had allowed the escape of ^{40}Ar . It is not usually possible to detect these effects by examination of the rocks in hand specimen or thin section. For instance, although Suess (1948), consulted "specialists for salt mines" his analyses demonstrated that

the samples he had been given had been recrystallised in recent times (Houtermans, F. G. in *Potassium-Argon Dating*, 5 Springer-Verlag; 1966). As a result the evaporite minerals have not subsequently been routinely employed for K-Ar dating.

The apparent ease of escape of radiogenic daughter isotopes from salt formations, particularly in recent times, and the alarming implications in the event of a containment failure, seem to require a thorough re-evaluation of the stability of these beds. Before any final decision on high-level radioactive waste disposal is reached, a systematic programme of argon measurements might provide a simple means of determining the extent of this phenomenon and enable its significance to be assessed.

R. M. MACINTYRE
University of Strathclyde, Glasgow

Alcohol in Britain

SIR,—J. A. Spring and D. H. Buss's account of three centuries of alcohol in the British diet (*Nature* **270**, 567–72; 1977) gives good evidence for the high intake of beer in Britain in years gone by. But may I add a comment to the statement that "there are no obvious reasons" for the steady decline in beer consumption during the eighteenth century (p 567)? It is that the increase in tea-drinking in that period was very much greater than taxation figures suggest.

Genuine China tea remained expensive throughout, but a great deal of what was consumed was in fact adulterated with the dried and chemically treated leaves of such trees as hawthorn, sloe and ash (known in the trade as 'smouch'), or with used tealeaves purchased at small cost from the kitchen-maids of the wealthy. The cheapest teas contained little else.

Tea-drinking increased steadily during the century in all classes of society, especially among women. For the poorest families in the South and Midlands, there was a reason for this increase. Enclosure and the high cost of fuel deprived them of soups and the cheapest cuts of meat, which required long cooking. Crude tea without milk was the only warm comforting element in a diet that otherwise consisted of bread, cheese and occasionally cold, fat bacon.

The changeover from beer to tea as the common mealtime drink was well under way during the 1730s and 1740s. And in 1784 the high import duty on tea was at last lowered to a nominal sum on the grounds that "tea has become an economical substitute to the middle and lower classes of society for

malt liquor, the price of which renders it impossible for them to procure the quantity sufficient for them as their only drink".

C. ANNE WILSON
*The Brotherton Library,
Leeds*

Dietary lipids and heart disease

SIR,—May I point out some fallacies in Dr John Rivers' review (*Nature* **270**, 2; 1977) of the so-called 'lipid hypothesis' of coronary heart disease (CHD)?

●The Finnish trial of Miettinen *et al* did show a notable decrease in mortality for males on a diet relatively high in poly-unsaturated fat. During the transcription of the mortality data from the original paper (Miettinen *et al*, *Lancet* **2**, 837; 1972) to the article discussed by your reviewer (Mann, G., *N. Engl. J. Med.* **297**, 646; 1977) an error was made which obscures the difference in mortality between the experimental and the control period ($32.00 + 2.84$ does not equal 38.84). It is disappointing that neither the editor of the *N. Engl. J. Med.* nor your correspondent noticed this.

●Unlike cigarette smoking, atmospheric pollution has not been shown to be an independent risk factor for CHD.

●Poly-unsaturated margarines do not necessarily contain *trans* fatty acids. The most popular Dutch brand of poly-unsaturated margarines contains 60 to 65% *cis-cis*-linoleic acid and 0% *trans* fatty acids. Its British equivalent ('Flora') has 6% of its fatty acids as *trans* isomers, which is similar to the proportion found in butter (data by courtesy of Unilever Research, Vlaardingen, The Netherlands).

●Epidemiological data overwhelmingly point in the direction of a relation between consumption of saturated fat and CHD (Keys A., *Atherosclerosis* **22**, 149–192; 1975). The Masai are only a notable exception. Incidentally it should be stressed that cause-effect relationships cannot be proved or disproved by such comparisons between tropical and western populations, but only by controlled experiments.

●Few people with original or heretic ideas in the food-health field are finding it really difficult to obtain research funds or publicity. Dr Mann himself, as the recipient of a career investigatorship from the National Heart, Lung and Blood Institute, is a case in point.

Nutrition is a billion-dollar business, a favourite subject for the media and an almost religious pre-occupation for a large part of the public. In view of these pressures, scientists bear a heavy responsibility to keep their data straight. In my opinion neither Dr

Mann nor your correspondent have succeeded in this.

MARTIJN B. KATAN
*The Agricultural University,
Wageningen, The Netherlands*

Intelligence is more than IQ

SIR,—I was interested in the letter by Brian J. Ford (5 January, page 7). I agree very fully with him of course, that the controversy is confused by the impossibility of obtaining measurements independent of cultural background. It is a great pity that so much more ink is being spilt in trying to prove that the heredity factor is unimportant, which for all I know may be true, than in the effort to raise all cultural backgrounds to an optimum level.

I would like to amplify Mr Ford's comment on the need for producing criteria which would reconcile mental measurements with the realities of life. What is practically accepted as intelligence depends to a very much more limited extent on IQ than is usually recognised. Thus IQ measurements often depend largely on the accurate understanding of the exact meanings of words and on the solving of logical problems. Solving real practical problems may often involve no language at all and in nearly all cases is concerned with the realities of a complicated situation rather than with the exact words by which this should be described. Furthermore the problems presented are unnatural in that the examinee knows that he has all the data required and that every datum is correct.

In real-life problems one never knows in advance whether one has all the necessary data and often is unsure whether the available data are right. Practical intelligence is therefore concerned with the judgement of whether sufficient data are available, with an understanding of how to obtain fresh data when necessary and then assessing the correctness or otherwise of the vital parts of the data available. This will involve persistence—in few practical situations must one solve 12 problems in 36 minutes—an ability to avoid prejudice, and some capacity for lateral thinking as well as IQ. Having satisfactorily solved all the practical problems the logical problem is nearly always trivial.

While a high IQ is valuable in a number of ways and probably has some correlation with the other factors I have mentioned, it is most unfortunate that it is so widely regarded as more important than it is, merely because it is so easy to put it in numerical terms.

J. H. FREMLIN
The University of Birmingham, UK

news and views

H-2 and HLA antigen structure

from Jonathan Howard

THERE have recently been remarkable developments in understanding the structure of the antigens of the major histocompatibility complex (MHC). For a variety of reasons the structures of these molecules are of consuming interest. First, they are powerful antigens; T cell responses to MHC antigens are one or more orders of magnitude stronger than other antigens. So abundant are T cells reacting to MHC antigens that it is at least debatable that the entire T cell pool is committed to the recognition of these structures.

Serologically the MHC antigens are exceedingly complex; each molecule, particularly those specified by the human HLA-A and HLA-B genes, and their mouse homologues H2-K, and H2-D, carries multiple antigenic determinants which constitute in sum the families of specificities that identify each MHC product. This serological complexity implies a genetic polymorphism of unprecedented extent. The presence of multiple alloantigenic specificities on a single molecule probably means that there is not just a single highly polymorphic site but several independently polymorphic regions on each molecule. Comparative sequence analysis of H-2 and HLA molecules available for the past couple of years (see *News and Views* 261, 189; 1976) suggests that the antigenic differences between H-2 and HLA allotypes reflect multiple amino acid substitutions at least at the NH₂-terminus, and tryptic peptide analysis of whole H-2 molecules shows that amino acid variation between allotypes must extend throughout a large portion of the molecule (Brown *et al. Biochemistry* 13, 3174; 1974). The origin and maintenance of such complex allotypes is a genetical problem of considerable interest in its own right (Silver & Hood in *Cont. Topics molec. Immun.* 5, 35; 1976).

MHC structures apparently corresponding to those identified as polymorphic antigens are also involved in the recognition by T cells of conventional

foreign antigens (Munro & Bright *Nature* 204, 145; 1976; Doherty *et al. Transplant. Rev.* 29, 89; 1976). Recognition of self MHC structures in either a native or altered form is essential for the induction and expression of specific T cell function to other antigens: this phenomenon of 'MHC restriction' implies either that T cells carry separate receptors specific for 'self' MHC structures and for foreign antigen (the 'dual recognition' hypothesis), or alternatively, foreign antigen is captured on the cell surface and presented to reactive T cells in some form of molecular association with self MHC structures (the 'altered-self' hypothesis).

It has been known for some time that the HLA-A and B molecules, and H-2K and H-2D molecules are physically associated with β_2 -microglobulin, a polypeptide with a molecular weight of 12,500 which in both sequence and intrachain disulphide bridge structure is homologous with a single domain of immunoglobulin. This has provoked speculation that the antigenic polypeptide chain of H-2 and HLA molecules might itself have sequence or structural homology with immunoglobulin, although this was not convincingly demonstrated by the first NH₂-terminal sequences that became available.

Detailed structural studies, primarily on the HLA-B7 molecule, by Strominger and colleagues (Strominger *et al. Proc. 3rd Int. Cong. Immun.* 1977, in the press) have given extraordinary new insights into the molecular organisation of a major transplantation antigen. Most striking of all is the unambiguous demonstration that this molecule does indeed have significant sequence and structural homology with immunoglobulin. This leaves little doubt that, as has been suggested before, the two molecular systems have had an, albeit distant, common precursor. The association of HLA with the Ig-domain-like β_2 M polypeptide may therefore legitimately be considered in the same context as the association of the evolutionarily divergent but homologous light and heavy chains of immunoglobulin.

The HLA-A and B molecules are

typical transmembrane glycoproteins with a molecular weight of about 45,000 having a strongly hydrophilic C-terminal intracellular segment, a short hydrophobic segment presumably responsible for membrane insertion, and a long extracellular hydrophilic segment representing about 80% of the molecule. The extracellular segment is folded into two non-intercalated disulphide-bridged loops and a free N-terminal segment. Each intrachain loop encloses a segment of approximately the same length as the intrachain loops of Ig domains. Sequence determinations near both the disulphide bridges reveal marked homology with immunoglobulin V_H sequence around the disulphide bridge at residue 95. Furthermore the N-terminal sequence of HLA-A and HLA-B, although not itself looped by a disulphide bridge, shows a significant homology with the same V_H region after appropriate alignment.

It is difficult to escape the conclusion that the extracellular portion of HLA is composed of at least two and possibly three mutually homologous domains, all showing significant homology with immunoglobulin, and two of them looped by disulphide bridges as in Ig. A peculiar aspect of the sequence homology between HLA and the V_H of Ig is that the relevant sequence runs from the C-terminal Cys of the first intrachain disulphide bridge toward the N-terminus in the V_H, and is thus entirely included in the looped segment; in HLA the homologous sequences run from the N-terminal Cys of the two intrachain bridges towards the N-terminus, and are thus entirely excluded from the looped segments. This important difference may well indicate that the similarity in domain structure between the two classes of molecule is more apparent than real; nevertheless the overall appearance remains of two, however distantly, related molecular species.

Although the residues responsible for the serologically detectable alloantigenic activity of H-2 and HLA molecules have not been identified, it is tempting to relate the extensive sequence variation throughout the molecules to the newly

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recognised domain structure and argue that the alloantigenic complexity of the molecules is due to independent sequence variation in each domain. In this way it is possible to have unique antigenic specificities (private antigens) associated in different molecules with various clusters of other specificities (public antigens). It may be anticipated that sequence variation associated with serological activity will be found to be spread over many parts of the molecule.

It seems unlikely, however, that allo-antigenicity is the most significant aspect of H-2 and HLA molecules for all its genetical and clinical importance. The recent discovery of H-2 and HLA restriction suggests that polymorphic structures on these molecules play an important part in T cell induction by antigen in the normal animal. Of particular interest therefore, is the relationship between the structure of the molecules involved in restricted interactions and the process of restriction itself. The mouse H-2K^b mutant H-2K^{ba} is a variant of considerable importance because, with respect to H-2 restricted lysis of virus-infected cells the mutant H-2 molecule is essentially non-overlapping with the wild type. T killer cells raised against virus-infected cells in the wild type H-2K^b mouse will lyse appropriately infected H-2K^b targets but not infected H-2K^{ba} targets, and *vice versa*. Comparison of the structure of H-2K^b and H-2K^{ba} molecules by Nathenson and

colleagues (*Cold Spring Harbour Symp. quant. Biol.* **41**, 343; 1977) has shown a limited number of peptide differences, compatible with a single amino acid substitution.

Furthermore, preliminary alignment of the variant peptides in the tentative model for H-2K (which is less developed than that of HLA-B7) suggests that they lie in or near the first disulphide-bridged domain. A location partially overlapping with H-2^{ba} is suggested for the non-identical but similar variant H-2K^{ba}. These findings indicate that the specificity of H-2 restriction is determined by a relatively limited part of the H-2K molecule. Interestingly, the variants H-2K^{ba} and H-2K^{bd} are not yet distinguishable from H-2K^b by conventional serological techniques, yet both variants provoke the full range of T cell responses by the parental type. It seems, therefore, that the mutated sites in these two variants are different from the predominant antigenic sites recognised by alloantibody.

Such observations have important implications for altered self or H-2 modification theories. The fact that the differences between H-2^{ba} and H-2^{bd} lie in a specific segment of the H-2 molecule implies that this segment must be important in the process of structural modification of H-2 antigen. Since the principal obstacle to the acceptance of an H-2 modification theory of H-2 restriction is the lack of any molecular mechanism by which antigen-specific modification might

occur, the localisation of a restriction site in a molecule whose structural organisation is beginning to be known should obviously be taken very seriously.

For the time being, the domain structure, association with β_2 -microglobulin and Ig sequence homology of H-2 and HLA seem significant without carrying a clear message. Without evidence for clonal allocation of different H-2 or HLA molecules between lymphocyte subpopulations, there are no grounds for believing that the specificity of antigen recognition by lymphocytes is due to sequence variations in these molecules. Nevertheless the apparent similarity between the two molecules may suggest that H-2 and HLA molecules are receptors for something at the cell surface. An analogy might be drawn with the two polypeptide hormones insulin and relaxin which, markedly similar in conformation, and plainly homologous, have entirely different biological roles while both function as hormones. I find it tempting to consider the possibility that the structure of H-2 and HLA molecules will indeed prove to be related to the phenomenon of MHC restriction by structural modification. However that may be, it is clear that the primary functions of these extraordinary molecules must be sought in the membranes into which they are inserted, rather than in their remarkable but probably biologically irrelevant immunogenicity in allogeneic responses. □

Invertible DNA in phage Mu

from Martha M. Howe

INVERTIBLE DNA sequences in prokaryotic DNA were first detected in the early 1970s during analysis of DNA from bacteriophage Mu. Since then, the discovery that inversion of a DNA segment controls the phase variation of flagellar antigens in *Salmonella* (Zieg *et al.* *Science* **196**, 170; 1977) and that inversion of an insertion sequence affects expression of the *gal* operon in *Escherichia coli* (Saedler *et al.* *Mol. gen. Genet.* **132**, 265; 1974), have lent credence to the hypothesis that inversion of DNA segments may be a general mechanism for the control of gene expression. Only recently has the role of DNA segment inversion in Mu development and gene expression begun to be understood.

An invertible DNA sequence in Mu was discovered in several laboratories studying the DNA forms arising after denaturation and reannealing of Mu DNA extracted from the mature phage

particle (Bade *J. Virol.* **10**, 1205; 1972; Daniell *et al.* *Virology* **51**, 237; 1973; Waggoner *et al.* *Proc. natn. Acad. Sci. U.S.A.* **71**, 1255; 1974). The two structures observed are shown in Fig. 1. One form was completely double stranded except for non-hybridizing single strands—'split ends'—found at the right end of the molecule. These split ends contain host DNA sequences which differ in different phage particles (Daniell *et al.* *J. Virol.* **15**, 237; 1975). The second form contained not only the split ends but also a single-stranded bubble structure termed the G bubble, flanked on its left and right by duplex DNA, the α and β segments, respectively. Hsu and Davidson (*Virology* **58**, 229; 1974) demonstrated that this G bubble arose by reannealing of Mu DNA strands containing the G segment DNA in opposite orientations. They postulated that inversion of G segment DNA occurred by recombination between two short inverted repeat sequences located at its ends and that the inversion was catalysed by a phage-specified function

active in both prophage and vegetative states.

One puzzling observation made by Daniell *et al.* (*Proc. natn. Acad. Sci. U.S.A.* **70**, 2153; 1973) was that the relative amounts of the two orientations differed in different phage populations. In DNA from lysates grown by induction of a lysogen both orientations of the G segment were present in approximately equal amounts; however, in lysates grown by infection 99% of the DNA had the G segment in one orientation, and this orientation was the same in all DNAs from lysates. The reason for this difference was not understood until experiments of Chow *et al.* (*J. molec. Biol.* **113**, 591; 1977) suggested that phage containing the G segment in opposite orientations might differ in their viability. This hypothesis was based on analysis of two classes of viable Mu phage containing partial deletions of the G and β DNA segments. Phage in one class were deleted for part of both the G and β segments and were unable to invert the G segment, pre-

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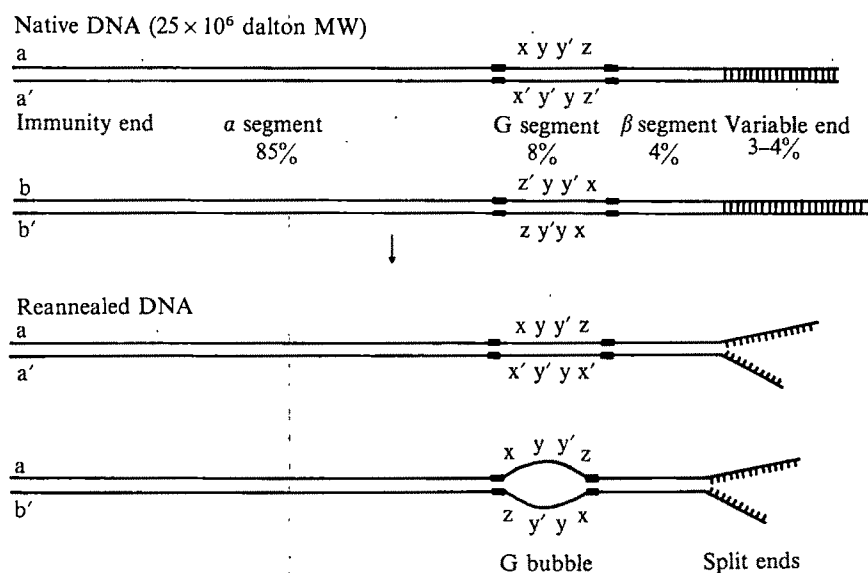


Fig. 1 Structure of Mu DNA. Structures observed in electron micrographs of denatured and reannealed Mu DNA. The $xyy'z$ and complementary $x'y'yz'$ lettering in the G segment does not represent specific genes (Hsu & Davidson *Virology* 58, 229; 1974). The black boxes flanking the G segment represent the inverted repeat sequences which serve as the sites of recombination for inversion.

sumably due to loss of one of the inverted repeat sequences, demonstrating that the process of inversion of the G segment itself was not essential for phage viability. Phage in the second class had an intact G segment and were only deleted for part of the β segment, yet they were also unable to invert the G segment. Chow *et al.* (*op. cit.*) postulated that phage in the second class were defective; in the Mu-specific function needed to invert the G segment, which they termed the *gin* (G inversion) function. It was the unexpected finding that both classes of phage contained the G segment in the same orientation as that predominating in lysates grown by infection that led to the hypothesis that phage containing the G segment in the opposite orientation might be non-viable due to lack of expression of essential genes located within the G segment DNA. Further experiments in their own and other laboratories have proved the validity of these hypotheses.

Symonds and Coelho (*Nature*, 271, 573; 1978) find that in single burst experiments with non-deleted heat-inducible Mu lysogens only half the cells released viable plaque-forming phage on induction. This is expected if half the cells contained Mu prophages with the G segment in the non-viable orientation. Kamp *et al.* (*Nature*, 271, 577; 1978) confirm and extend this finding by testing populations of cells lysogenic for a heat-inducible Mu prophage for their relative ability to release plaque-forming phage on induction and for the orientation of the G segment in DNA extracted from phage particles produced as a result of induction. They found that the proportion of cells able to release plaque-forming phage was directly correlated with the proportion of phage DNA containing the G segment in the viable orientation. In a second experiment they demonstrated that

a Mu *gin*⁻ prophage could be complemented for inversion of the G segment by transient exposure of the cell to a Mu *gin*⁺ prophage. Two types of Mu *gin*⁻ lysogens resulted. The first released plaque-forming phage particles whose G segment was in the original viable orientation. The second released phage particles which could not form plaques and whose DNA contained the G segment in the opposite or non-viable orientation. Thus they have definitively demonstrated that the orientation of the G segment affects the viability of the phage particle.

Experiments by Bukhari and Ambrosio (*Nature*, 271, 575; 1978) reveal that the phage with the G segment in the wrong orientation are non-viable because they cannot adsorb to cells, whereas phage with the G segment in the viable orientation adsorbed to cells and replicated their DNA normally.

Mu genes whose expression might be determined by the orientation of the G segment have been identified in our laboratory. We have found that the essential genes *S* and *U* of Mu map within the invertible G segment. Although the functions of these genes have not been conclusively determined, several lines of evidence suggest that they may be involved in adsorption. First, serum blocking experiments indicated that the phage inactivating activity in anti-Mu serum was directed against the *S* protein of the phage particle. Second, Mu *S*⁻ and *U*⁻ phage which acquired an *S*⁺ or *U*⁺ phenotype by recombination with phage P1 often simultaneously acquired the host range of P1 (Toussaint *et al.*, private communication). These recombinants probably arose through exchange in the regions containing the G segment of Mu and a similar invertible segment of P1 which has extensive homo-

logy to the Mu G segment (Chow & Bukhari *Virology* 74, 242; 1976) and which may determine the host range properties of P1 (Toussaint *et al. op. cit.*).

The experiments described above indicate that the orientation of the G segment of Mu determines the expression of adsorption functions of Mu, perhaps by controlling the synthesis of the *S* and *U* proteins. Why should such relatively unrelated phages as Mu and P1 have homologous invertible segments and why should Mu have an invertible segment whose inversion makes the phage particle non-viable? Possible explanations were discussed at the Bacteriophage Meetings held at Cold Spring Harbor Laboratory last August. Normal genetic exchange by homologous recombination between ancestors of Mu and P1 might explain the invertible segment homology in the two phages; however, the lack of homology elsewhere in the phage suggests the intriguing possibility that the invertible segments may be able to translocate independently from one DNA to another. In this case Mu and P1 might have acquired the segment from a common source, or the segment present on one of the phages might have translocated to the other. The different lengths of the inverted repeat sequences of the elements in two phages in the translocated element suggests that changes may have occurred subsequently (Chow & Bukhari *Virology* 74, 242; 1976).

Why does Mu possess a function which impairs its viability. It may be pertinent to consider the organisation of the control system. Two possible simple on-off control mechanisms might exist. One is that postulated for the control of antigenic phase variation in *Salmonella* (Zieg *et al. Science* 196, 170; 1977) in which the promoter is present on the invertible sequence and the structural gene under its control is not. The second, perhaps more applicable to Mu G segment inversion, is one in which the structural genes are located in the invertible segment with the promoter outside. In both these cases the synthesis of the structural gene products would depend on the orientation of the invertible segment. A third possible organization is one in which the invertible segment may contain genes at each end which specify different adsorption proteins responsible for determining the host range of the phage. If the promoter for these genes were located outside the invertible segment such that only the genes immediately adjacent to the promoter were expressed, inversion of the segment might lead to expression of a different host range. The ability of the phage to alternate between two different host ranges might provide it with a selective advantage in natural populations. Such a model might be directly applicable to P1 since P1 DNA contains equal amounts of both orientations regardless of its mode of growth (Ohtsubo,

cited in Chow & Bukhari *Virology* **74**, 242; 1976). To apply the model to Mu it would only be necessary to postulate either that Mu might be defective in the second set of genes or that Mu phage with the G segment in the non-viable orientation are able to adsorb to other

bacterial strains which have not yet been tested. Further experiments will more completely elucidate the role of G segment inversion in expression of Mu genes and will reveal whether inversion of DNA segments comprises a general mechanism of gene control. □

How plants respond to stress

from Hamlyn G. Jones

THE plant hormone abscisic acid (ABA) has been invoked to explain a host of plant processes ranging from dormancy and leaf abscission, through flowering and fruit drop to the response of roots to gravity. However, it is as a stress hormone involved in the drought-induced closure of stomata, that there is the most complete evidence for a natural role for this hormone. The stomata are the chief control on water loss from plants, so that stomatal closure is an important mechanism enabling plants to survive in dry environments.

There is now evidence that the amount of ABA in tissues of many plant species increases rapidly when the plants are subjected to various stresses, including water deficits. Increased concentrations of ABA can be detected within 7 minutes of the start of a stress period and can increase as much as 40-fold over 4 hours. The evidence that this stress-induced ABA is involved in at least some of the natural stomatal movements is now overwhelming. Not only is the amount of ABA extractable from leaves generally related to stomatal aperture, but ABA applied to leaves usually results in rapid stomatal closure. Further evidence comes from work by Tal and coworkers at the University of the Negev on a wilted mutant of tomato (*flacca*) which does not close its stomata. This lesion was found to be due to a deficiency of ABA which could be overcome by supplying extra hormone.

Two papers in this issue of *Nature* (Itai *et al.*, page 652 and Itai & Meidner, page 653), provide further evidence of a role for ABA in stomatal physiology. These workers demonstrate, using microautoradiography of leaf or epidermal tissue supplied with ^{14}C -labelled ABA, that the hormone can accumulate at the presumed site of action—the stomatal apparatus.

This extends recent work by Loveys (*Physiol. Plant.* **40**, 6; 1977) who showed that although ABA does not appear to be synthesised in the epidermis it does accumulate in the epidermis

under stress. Nevertheless, there has not yet been an unequivocal demonstration that ABA accumulates in the stomatal apparatus during natural stress.

If the details of ABA accumulation are not yet proven, the mode of action of the hormone is even less certain. Changes in stomatal aperture are directly caused by changes in the balance between the internal pressure of the guard cells (specialised cells which surround the stomatal pore) and other epidermal cells. The high pressures necessary to open stomata arise from the active accumulation of osmotically active molecules and ions, especially K^+ . It is likely that ABA acts in some undetermined manner on the accumulation or retention of K^+ , as discussed by Itai and Meidner.

In spite of these recent successes in explaining stomatal movements in terms of ABA it is as well to remember that this is not the only mechanism involved in controlling stomatal movements. In the first place, so-called hydroactive closure can begin within a few seconds of changes of leaf water status; a response which is too rapid to involve a hormone such as ABA. Second, the hormone is probably not involved in the normal diurnal opening and closing movements found in most plants. Third, there are several reports of the involvement of other naturally occurring compounds in the regulation of stomatal aperture, particularly under stress. Most evidence has been adduced for an involvement of all-*trans*-farnesol (a sesquiterpenoid like ABA) which Mansfield's group at Lancaster have reported (Ogunkanmi *et al. Planta* **117**, 293; 1974) to accumulate in drought-stressed sorghum and to be effective at closing stomata.

The question still remains as to whether the postulated role for ABA in promoting stomatal closure is the main role for the apparently excessive quantities of the hormone produced in stress conditions. Many of the known effects of ABA, including reduction in leaf growth or even enhanced leaf senescence (see Milborrow *A. Rev. Pl. Physiol.* **25**, 259; 1974) as well as the reduced proportion of epidermal cells which form stomata and the increased

production of epidermal hairs (Quarrie & Jones *J. exp. Bot.* **28**, 192; 1977), are also well known symptoms of water stress. In the long term, these changes, particularly the reduction in leaf area, may be as important as stomatal closure in reducing water use by plants. Other work (Glinka *Pl. Physiol.* **51**, 217; 1973) indicates that ABA can increase the rate of water uptake by plant roots. Taken together, these results suggest that ABA may be involved in a whole syndrome of responses, all of which may contribute to adaptation to stress environments.

Perhaps many of the other reported effects of ABA, such as those on flowering, may also prove to be adaptations to stress environments. Unfortunately much of the evidence on the developmental effects of ABA is contradictory, so there is a clear need for more careful studies of the effects of applied ABA, taking account of the quantities of the hormone getting into the tissue, its distribution and its ultimate fate.

Research on ABA may have important practical consequences. Following the demonstration that some drought-tolerant species or varieties produce more ABA than sensitive ones (Larqué-Saavedra & Wain *Nature* **251**, 716; 1974; *Ann. appl. biol.* **83**, 291; 1976), several groups around the world are currently screening agricultural crops for ABA production in attempts to breed new varieties capable of high yields in dry regions. Other work concentrates on the use of ABA or its analogues as anti-transpirants to be applied to crops when drought occurs. Much of this work involves the implicit assumption that stomatal closure due to enhanced ABA levels is advantageous in dry situations. Unfortunately the reduction in photosynthesis and harvestable yield which accompanies stomatal closure may outweigh any saving in water, while rapid stomatal closure does not always appear to improve drought tolerance in crops. □

The man-made mouse

from John K. Heath

TERATOCARCINOMAS have been the subject of intensive research effort over the past few years. They are malignant tumours composed of 'undifferentiated' embryonal carcinoma stem cells (EC cells) which can differentiate *in vivo* or *in vitro* into apparently normal differentiated tissue. Since the cells can grow and

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differentiate in culture they could provide a large scale source of material whose behaviour mimics that of the relatively inaccessible mammalian embryo.

The teratocarcinoma story took on a new perspective when it was established by Brinster (*J. exp. Med.* **140**, 1049; 1970), Illmensee and Mintz (*Proc. natn. Acad. Sci. U.S.A.* **72**, 3585; **73**, 549; 1975) and Papaioannou *et al.* (*Nature* **258**, 70; 1975) that when an EC cell from a teratocarcinoma was injected into a normal mouse blastocyst, it could differentiate into fully functional, non-malignant, normal mouse tissue. The possibilities opened up by these observations are enormous. One can suppress the malignancy of an EC cell by placing it in a normal developmental environment. It might be possible to select mutations in culture which are not currently available in normal mouse stocks and examine their effect *in vivo*, thus generating mouse models of human genetic disorders. The ultimate goal would be to achieve a form of genetic engineering at the whole organism level by incorporating genetically manipulated EC cell derivatives into the germ line of a normal mouse and so produce mutant mouse stocks created *in vitro*; the man-made mouse.

Two important advances towards this goal have been achieved. Papaioannou and her colleagues (*Nature*, *op. cit.*; *J. exp. embryol. Morph.* in the press) have shown that EC cells from some cultured teratocarcinoma cell lines can also form functional differentiated tissue in a mouse injected with the cells at the blastocyst stage. This observation has been extended by Dewey *et al.* (*Proc. natn. Acad. Sci. U.S.A.* **74**, 5564; 1977) who have been able to produce a number of mice containing tissues derived from mutant injected cells. The EC cells in question lacked the X-linked enzyme hypoxanthine phosphoribosyl transferase (HPRT). This is a good choice of mutation because it is not available in mouse stocks, but can be readily obtained in cultured cells. HPRT deficiency produces a severe neurological disorder, Lesch-Nyhan syndrome, in humans. A deficiency of this enzyme in humans can also lead to some types of gout. Ten of the mice which have been analysed so far in this study have been shown to contain tissues derived from the donor EC cell. These workers have further been able to demonstrate that the EC cell's derivatives in the normal mouse retain their enzyme-deficient phenotype. None of the chimaeric mice has Lesch-Nyhan symptoms however, despite extensive colonisation of the nervous system by donor tissue in some cases. One further point of interest is that teratocarcinoma-derived blood tissue is rare in these chimaeras, when compared with chimaeras containing tissues from the wild-type version of the same cell line. This could possibly be due to the fact that HPRT⁻ blood cells are at a selective

disadvantage in the normal mouse circulation. This compares with human HPRT⁻/HPRT⁺ female heterozygotes where, due to X-chromosome inactivation, blood cells express either the HPRT⁻ or the HPRT⁺ phenotype, and this has been shown to result in a selective advantage for the HPRT⁺ cells, resulting in an overall HPRT⁺ blood phenotype (Nyhan *et al. Proc. natn. Acad. Sci. U.S.A.* **65**, 214; 1970).

Although the results so far have seemed very promising, a number of important problems have emerged. If a case is to be made for the use of teratocarcinomas as a way of studying normal development, either on their own or in chimaeric combinations with normal mouse embryos, it must be established that the behaviour of an EC cell in a blastocyst parallels that of its normal embryonic equivalent. On the basis of the available evidence, EC cells cannot be said to be the equivalent of the inner cell mass (ICM) cells of the normal embryo. If ICM cells are injected into a mouse blastocyst the resulting chimaeric adult has contributions from the injected cell in nearly all the tissues that have been examined (Ford, Evans & Gardner *J. Embryol. exp. Morph.* **33**, 447; 1975). In addition the chimaeras obtained from ICM injections show a very fine-grained intermingling of donor and host tissue (this is particularly noticeable in the coat, for example). In general, chimaeras from injected EC cells have EC contribution in few tissues, and the EC cells tend to occur in much larger patches compared with ICM cell chimaeras.

Another problem, especially in view of the potential practical applications of these animals in the study of human disease, is that there is only one published report of EC cells differentiating into functional gametes (Mintz & Illmensee *Proc. natn. Acad. Sci. U.S.A.* **72**, 3585; 1975). In this case the EC cells were taken from an *in vivo* ascites teratocarcinoma. No germ-line chimaeras have been reported from blastocysts injected with EC cells from cultured teratocarcinoma cell lines. This is not encouraging, especially when this one animal is set against the total number of blastocysts injected with EC cells. It is not yet clear why most EC cells fail to form functional gametes. One possibility is that most of the teratocarcinoma lines which have been tested are karyotypically abnormal. It is known that most gross karyotypic changes in the normal mouse result in sterility, and it is conceivable that the germ line has some form of 'self defence' mechanism to prevent the inheritance of deleterious chromosomal abnormalities. This problem could be studied with the use of a number of karyotypically normal teratocarcinoma cell lines which have been established.

Another difficulty is that there is no way of controlling the sort of tissues that an

EC cell will form in a normal embryo. This means that comparisons between human disorders and teratocarcinoma chimaeras are very difficult, unless one tissue alone, by chance, happens to be derived in part from the donor EC cell.

The use of teratocarcinoma cell chimaeras as a practical tool in developmental biology and medicine has enormous potential. The results in this area are exciting, but a lot more information is needed on the nature and behaviour of EC cells in the normal mouse embryo before the 'man-made mouse' takes its place as a standard laboratory research tool. □

Beads and skeletons

from Graham Cowling

THE dramatic structural changes that occur in the nucleus of a cell as it prepares to divide, have fascinated biologists for over a century and yet, in terms of molecular interactions, the structure of the eukaryotic chromosome is still far from understood. The revealing discovery of beaded chromatin structure has led to rapid progress in the study of DNA-histone interactions. These advances have, in turn, resulted in various models being proposed to explain how arrays of nucleosomes, the basic building blocks made up of DNA and histones, can be supercoiled to form the higher order structures seen *in vivo* (see for example, Finch & Klug *Proc. natn. Acad. Sci. U.S.A.* **73**, 1897; 1976). Are these aggregates of nucleosomes sufficient to give a complete understanding of the overall organisation of chromatin in chromosomes? It seems unlikely from the recent results of U. K. Laemmli's group which suggest that a network of non-histone proteins is responsible for organising chromatin in metaphase, and possibly interphase, chromosomes.

Laemmli and coworkers (*Cell* **12**, 805; 1977) showed that, after histones and the majority of non-histone proteins had been removed from HeLa metaphase chromosomes by treatment with dextran sulphate and heparin, the chromosomal DNA remains in an organised and reasonably compact structure. These histone-depleted structures sedimented in sucrose gradients as a broad peak (4,000–7,000 S) at a density range distinct from free DNA or intact chromosomes. The isolated complexes, stained with ethidium bromide, appeared under the fluorescent

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microscope as a central core with the morphology of intact chromosomes, which was surrounded by a halo of DNA. Each chromatid was still paired with its sister chromatid although the central structure was 2–3 times the size of the original chromosome. These histone-depleted structures could be dissociated in 4 M urea, but remained intact when isolated in gradients containing 2 M sodium chloride. Mild treatment with trypsin or chymotrypsin also destroyed their morphology.

Gel electrophoresis of the proteins in the histone-depleted chromosomes revealed the presence of about 30 non-histones, most of which had molecular weights greater than 50,000. No bands with the mobilities of either histones or actin, previously found in nuclei (Douvas *et al. Proc. natn. Acad. Sci. U.S.A.* **72**, 3902; 1975), were seen but one of the three major bands had a molecular weight close to that of the related fibrous protein β -tubulin, but as yet not identified as such.

Electron micrographs of these histone-depleted metaphase chromosomes (Paulson & Laemmli *Cell* **12**, 817; 1977) show in more detail how the core of condensed material retained the original chromosomal morphology and was surrounded by many loops of DNA. In some pictures individual DNA loops, ranging in size from 30–90 kilobases, could be seen leaving the core and returning to a position adjacent to their exit points. No super-coiling of the DNA loops was seen, but as Paulson and Laemmli point out, the preparation of such complexes probably allows nicking of the DNA strands to take place. When histones were removed from the chromosome spreads by prolonged washing with 2 M sodium chloride, reported to remove all histones, similar structures to those prepared by polyanion methods were seen by electron microscopy. It therefore seems unlikely that traces of histones were responsible for retaining the morphology seen.

Concurrent work by Laemmli's group (*Proc. natn. Acad. Sci. U.S.A.* **74**, 4937; 1977) has shown that by treating intact HeLa metaphase chromosomes with micrococcal nuclease, a protein 'scaffold' independent of DNA can be isolated on sucrose gradients. The proteins in these scaffolds were identical to those found in the histone-depleted chromosomes. Even more surprising was that the scaffold's shape resembled that of the original metaphase chromosomes, with pairs of sister chromatids still attached by the edges of the scaffold. This suggests that a network of non-histone proteins rather than DNA governs the overall packaging of the chromosome. Another intriguing aspect of these nuclease-prepared scaffolds is that they contain

small pieces of tightly bound DNA. If scaffold proteins recognise a few specific sequences of DNA, considering the number of loops seen in these structures, any such sequences must be at least called repetitive. Perhaps this represents an exciting new system for the study of DNA-protein recognition mechanisms.

Previous models for chromosome structure have been suggested by electron micrographs of intact chromosomes and recently complemented by the study of the nucleosomal packaging of chromatin. In most part non-histones have been left out of models that use a central core of chromatin to organise the rest of the DNA fibres into loops around its axis (see Stubble-

field & Wray *Chromosoma* **32**, 262; 1971). Laemmli's group has proposed a new model which uses a network of non-histone proteins to both trace out the shape of the chromosome and to organise the surrounding DNA into loops. In histone-depleted chromosomes the masses of free DNA strands are probably the result of unravelling the solenoid or 'superbead' arrays of nucleosomes that surround the scaffold. If this proves to be a general model for metaphase and, possibly, interphase chromosomes further investigation of the scaffold proteins and their changes during the cell cycle may allow a greater understanding of how the cell organises replicated DNA before mitosis. □

Electrical conduction in discontinuous metal films

from Robert M. Hill

DISCONTINUOUS metal films deposited on insulating substrates have been of interest to solid state physicists for many years.

Nucleation theory supports the experimental observation that the initial condensate of a vapour on a clean surface is in the form of small particles and not as a uniform, constant thickness layer. This applies not only to water vapour on a cool substrate but to the deposition of metallic vapours under vacuum conditions. It is only when the average thickness of the deposit is more than some critical thickness that physical continuity is established within the metallic film. The structure of the deposit, in the initial stages of growth, is sensitive to many of the physical parameters of the system. A metal-substrate combination that gives a low surface mobility for the condensate will yield a film formed from small particles closely packed on the surface. Typical dimensions can be as small as 5 nm particle sizes and less than half this figure for the interparticle spacings. These dimensions can be varied by changing the physical conditions during deposition, a higher substrate temperature giving larger particles and wider spacings.

In the classification of solids as metals, insulators and semiconductors the quasi-two dimensional particulate film has been found, experimentally, to bridge the region between insulating behaviour, when the interparticulate spacings are large, and metallic behaviour, when the particles have grown sufficiently to aggregate

and form metallic, percolative, paths. Between these limits a semiconductor-like behaviour is seen but the activation energy of the current is generally very small, and usually less than 0.1 eV.

The transition between the semiconducting and metallic regions can be observed by a change in the sign of the temperature coefficient of resistance. The low temperature coefficient region is of obvious interest as an electronic circuit resistor. Three-dimensional particulate films can be prepared by the co-deposition from vapours of metallic and insulating components, the cermet film, and show properties similar to the two-dimensional structures, with an improved stability. The cermet structure has been used as resistors for almost 20 years.

As with most surface phenomena the properties of a discontinuous metal film are strongly dependent on the ambient conditions. Indeed the films are at best only metastable and can be annealed at temperatures in excess of the deposition temperature with a consequent change in the physical structure and hence in the structure-sensitive properties. This makes physical examination, and the correlation of the structures with other properties, difficult. In a recent review of the field Morris and Coutts (*Thin Solid Films*, **47**, 3; 1977) have critically examined the published information and reach the conclusion that the nature of charge transport in particulate films is, as yet, poorly understood. Much of the information is contradictory and in very few cases has sufficient detail been given to

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allow evaluation and comparison of the physical phenomena involved.

Initially the activated behaviour was taken to be evidence of thermal ionisation effects but the magnitudes of the energies involved were found to be roughly inversely dependent on the particle sizes. It is now accepted that coulombic interactions do form the basis of this energy but that the physical size of the particles cannot be neglected. The transport of charge from one particle to another can occur either through the insulating substrate or through the free space. Examination of the energies involved shows that the former is more probable, and the strong dependence of the resistance of the films on the particle spacings indicates that the mechanism is likely to be due to quantum mechanical tunnelling. Supporting evidence is found from the detailed temperature dependence of the low field conductance and from the electric field dependence of the current at high fields. However the magnitudes of the observed currents do not correlate with the observed structure. The obvious explanation of this discrepancy is that the structure is not uniform, and may not even be homogeneous. Electron microscopical investigations show that this is indeed the case, with wide distributions both of particle size and spacing. Only recently have attempts been made to introduce the distributed nature of the parameters into the theoretical models of transport (Abeles *et al. Adv. Phys.* **24**, 407; 1975; Hill & Coutts *Thin Solid Films* **42**, 201; 1977; Leaver *J. Phys. C* **10**, 249; 1977). The problem is undoubtedly difficult for it is necessary to use a percolative approach in which easy transport paths through the material are given a probability weighting dependent on their conductance. But even within these paths the individual particles and gaps are distributed. Recent work has shown that at high temperatures a clear activated region should be observed when transport is between nearest neighbour particles, but at low temperatures where the number of easy paths decreases the activation energy will become temperature dependent as carriers have to be transported to more distant neighbours. This second region of behaviour is similar to the variable range hopping region in amorphous semiconductors (Mott *Festkörperprobleme* **9**, 22; 1969) or in impurity band conduction in heavily doped and compensated semiconductors (Mott & Davis *Electronic Processes in Non-Crystalline Materials*; Clarendon Press, Oxford, 1971) except that the size of the active centres is much larger and hopping can only occur between the supply particle and a limited number of nearer neighbours. Experimental verification of the temperature dependence of the activation energy is not available yet as very few films have been examined at sufficiently low temperatures to observe the full transition in behaviour

(Abeles *et al. op. cit.*).

A range of interesting physical effects has been observed in discontinuous and quasi-continuous metal film structures (Morris & Coutts *op. cit.*). A number of these could form the basis of practical sensors but as electrical charge transport

is central to the understanding of these effects it is essential that our understanding of the process, or processes, involved should be placed on a firm foundation. In their review Morris and Coutts clearly show that this is not the case. □

New light on Phobos, the inner satellite of Mars

from G. Fielder

PHOBOS is a dynamic body with a difference: it is one of the smallest natural satellites in the Solar System, and completes one revolution of Mars every 7.65 hours at a mere 9,000 km from the planet's centre. Recent discoveries about Phobos have opened up exciting prospects for students of planetary science. First, close up views of intact small bodies in the Solar System are few; and, second, exploration of numerous planetary bodies (including natural satellites and asteroids) which offer, collectively, a range of size and a large spread of solar distance is of the highest importance in unravelling the early history and origin of the planetary system.

Two quantities—the radius and mass—are fundamental in planetology. Until the Mariner spacecraft photographed Phobos, in 1969 and (better) in 1971, its

mean radius had to be estimated by assuming an albedo and measuring the amount of sunlight that the satellite scattered back to Earth. And until the Viking Orbiter I was Doppler-tracked, in 1977, to measure its accelerations relative to Phobos, the mass of Phobos could not be computed. Once the mass and radius of a planet are known, the mean density can be calculated: that is important because it is related to the planet's composition. Phobian mass determinations, by two groups of researchers (Tolson *et al. Geophys. Res. Lett.* **4**, 551; 1977; Christensen *et al. Geophys. Res. Lett.* **4**, 555; 1977) who used different methods of processing the same raw data, are therefore of very great interest. The mean value is close to 1.0×10^{16} kg.

But Phobos is not a sphere. There is no reason why it should be since, on such a small body, the particles experience little in the way of mutual gravitational attraction. Photographs (Veverka *et al.*

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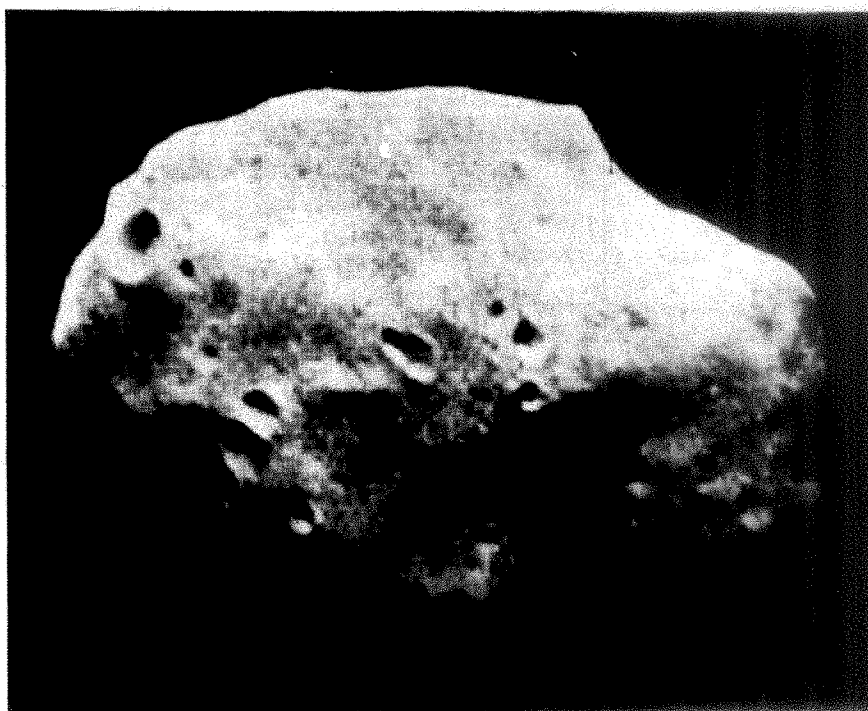


Fig. 1 NASA Mariner 9 photo of Phobos showing craters.

Icarus 23, 206; 1974) taken by Mariner 9 showed Phobos (Fig. 1) to be intensely cratered and highly irregular in shape. A few of the craters, such as the 10-km diameter Hall, have widths approaching the mean radius of Phobos itself, and these craters contribute markedly to the overall irregularity. Even a triaxial ellipsoid is not too good a fit to the surface of Phobos, which seems to have semi-axes of about 13.5 (pointing towards Mars), 10.7 and 9.6 km, respectively. The volume of Phobos, corrected for the volume of the craterial depressions (mainly, the largest ones) below the ellipsoid, is about $5 \times 10^{12} \text{ m}^3$; and thus the density turns out to be only $2,000 \text{ kg m}^{-3}$ (2 g cc^{-1}). It is a surprise because it is so small: some scientists view Phobos as a captured asteroid, and the mean density of asteroids is likely to be more nearly $3,500 \text{ kg m}^{-3}$.

What, then, can be deduced about the composition of Phobos? The albedo (Short *Planet. Geol.*, Prentice-Hall, 1975) of Phobos is similar to that of chondritic material, but this has a density that is higher than $2,000 \text{ kg m}^{-3}$. However, the observed low mean density of Phobos can be synthesised using a suitable mixture of carbonaceous chondritic material (which itself contains 20% by weight of water) and voids, or low density materials such as ice. Under the prevailing conditions (the surface temperature of Phobos may be calculated, using an albedo of 0.05, and attains some 230 K or -40°C) ice could exist inside the satellite provided that the interior was not warmer than predicted on the basis of solar heating alone. However, present-day heating of Phobos is not unlikely. Phobos actually resides within the Roche limit for a fluid body which, had it replaced Phobos, would have disintegrated. Phobos remains in one piece because it has more mechanical strength than a fluid. Yet, if the tiny moon were to move only 3,700 km closer to Mars, then even Phobos would probably be torn apart. With the present Mars-Phobos configuration, the materials of Phobos are being subject to stresses of the order of 10^4 N m^{-2} . This kind of stress level is insufficient to fracture solid rocks as we know them on Earth, but new Viking Orbiter photographs (Fig. 2) of Phobos show that its underdense materials have indeed been tidally fractured: the surface displays striking arrays of lineaments (actually approximating to arcs of ellipses), and these lineaments trend in just those directions predicted by the theory of tidal fracturing of a natural satellite which is gravitationally bound to face its primary (Gash, thesis, University of Lancaster, 1973). Even though the orbital eccentricity (0.02) of Phobos leads to rather small changes in the tide-raising force, the components of this force also change in magnitude as a result of the physical libration of Phobos (Duxbury *Icarus* 23, 290; 1974). An

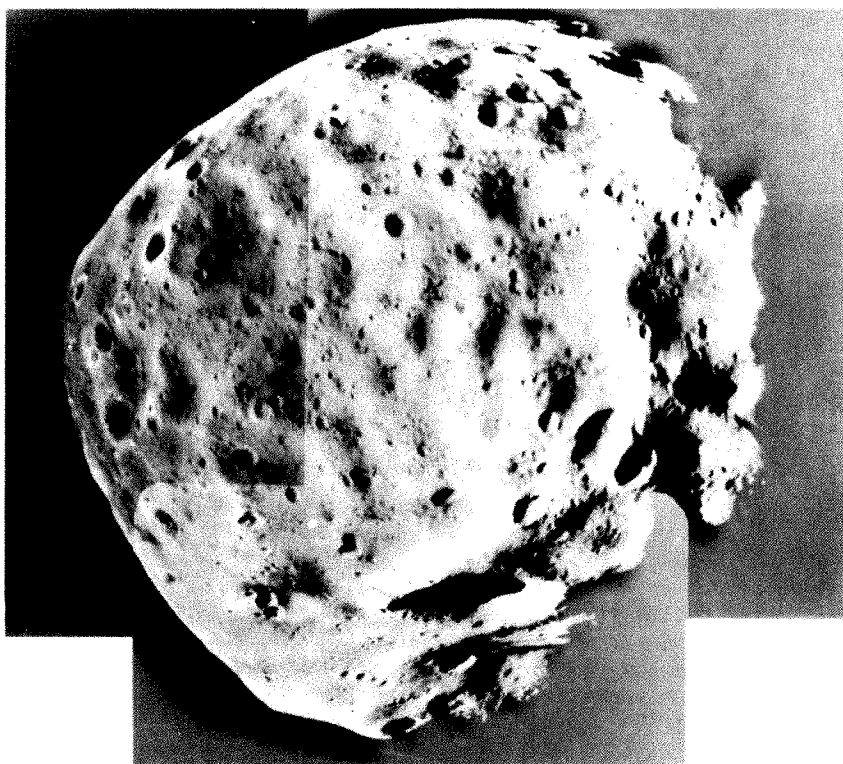


Photo: NASA

Fig. 2 NASA Viking Orbiter photo of Phobos showing crater chains. A series of craters runs horizontally in the picture, which is parallel to the orbit plane of Phobos.

effect of such tidal stressing is to dissipate gravitational energy as heat—in this case, mainly along the fracture lanes. The underdense rock conductivity will be small and it is conceivable that repeated stress changes will lead to the melting of the materials along the fractures. The volatiles will have left through the impact-pulverised upper layers of Phobos and the loosely bonded surface materials will have subsided into the vacant spaces to produce crateriform depressions along the

lengths of the fractures, as observed (Fig. 2). Since these lineaments, or crater chains, seem to dissect all the larger craters on Phobos, it is clear that the fractures have been worked more recently than the most important phase of impact cratering.

Further studies of the new data are likely to throw some light on the orbital evolution of Phobos. Whether or not Phobos is a captured asteroid may then become clearer. □



A hundred years ago

At the meeting of the Linnean Society on Thursday last it was unanimously resolved to send a congratulatory letter to von Siebold on the occasion of his jubilee. This graceful act, however, brings into prominence the neglect of the Society to take any notice of the Linnean centenary, the celebration of which in Sweden, Holland and Germany were recently noticed in our columns. Of course the excuse may be urged with some force that such formalities are foreign to English habits, but perhaps an exception might have been allowed in the case of a Society which bears the name and jealously guards the collections, books and manuscripts of the great naturalist. Perhaps however, another reason may be found in the fact that the constitution of the Society places the initiative in every case in the hands of

officers whose tenure of office is practically indefinite, and who are not very accessible to any impulses of enthusiasm from the general body of the Society even if there were any permissible way by which expression could be given to them. Some disquieting rumours as to the present condition of the Society's business affairs, coupled with its rather troubled history during the past few years, seem to point to the desirability of some changes in its mode of government which would bring the executive into closer relation with the general body of Fellows.

AMONGST the exports of Corsica it is said that there are annually between 350,000 and 400,000 blackbirds (*merles*) which are sent to this continent. They visit Corsica in vast numbers each winter to feed on the berries of the myrtle and arbutus, with which the mountains are covered. In the month of December they become very fat and the flavour and the perfume given to their flesh by their food causes them to be much esteemed by the *gourmets* of Paris. A *pâté de foie de merle* is a great delicacy.

From *Nature* 17, 14 Feb. 308, 309; 1878.

nature

16 February 1978

Prevention of spina bifida—the parents' choice

THE problem of prevention and treatment of the congenital malformation spina bifida is in the news again. An all-party motion in the British House of Commons to make prenatal screening for the condition available to all expectant mothers as soon as possible has been signed by over 200 MPs. The screening would involve a blood test, followed, if positive, by amniocentesis (drawing off amniotic fluid) to provide a more sensitive test, followed by offer of abortion if the foetus were found to have spina bifida. Amniocentesis is also of value in the detection of chromosome anomalies such as Down's syndrome and a number of other disorders. Some strong views and some incorrect statistics have been flying around this past month; maybe it will be of value first simply to record some of the basic facts.

The incidence of the neural tube defects, anencephaly and spina bifida varies with race and place. In south-east England the incidences are one in 800 births for anencephaly and one in 600 for spina bifida. But in Wales, Scotland and Ireland the incidence is roughly double this, whereas in African and Mongoloid races the incidence can be less than half that in south-east England. The reasons for this variation are unknown—a different gene pool, soft water or the consumption of blighted potatoes have all been suggested as causes, but so far without confirmation.

If parents have had an affected child the incidence in future siblings is around 4%. There is evidence that both genetic and environmental influences are at work; in most series, consanguinity in the parents is found to be commoner than in the average marriage, monozygotic twins show a higher concordance rate than dizygotic twins and incidence is higher in lower socio-economic groups.

The proposed screening test is an assessment of the level of alpha-fetoprotein in maternal serum. This alpha-immunoglobulin, of uncertain function, is synthesised by some foetal cells and the concentration in foetal serum rises from the 6th to the 14th embryonic week, after which it declines to zero at birth. In neural tube defects the amniotic fluid level is considerably greater than normal and this is reflected in a significant but lesser elevation of the level in the maternal serum. Unfortunately a number of other conditions can give unusually high alpha-fetoprotein levels in the amniotic fluid, such as twinning, foetal death, rhesus immunisation and some other congenital deformities, although it has recently been

shown that study of the cells in the amniotic fluid can discriminate between neural tube defects and other causes of raised alpha-fetoprotein. Other tests such as X-rays, ultrasonics and foetoscopy (direct inspection of the foetus through a fibre-optic instrument) can add to the accuracy of diagnosis, so the likelihood of a false positive result in experienced centres is small. The risk to the mother also seems to be very small, apart from the possibility of rhesus sensitisation. There is a risk estimated at 1–2% to the foetus, mainly of induced abortion, though a recent study showed no significantly increased incidence of abortion.

The outlook for a child born with a neural tube defect varies from certain death in anencephaly to mild weakness of the lower limbs when the defect is minimal. A typical case, if left untreated, will die of infection in due course (though there is occasional survival). With surgery, survival is usual, which is why this was undertaken for most cases from the late 1950s. But there is usually considerable handicap (paralysis of legs, incontinence and deformity—sometimes hydrocephalus and mental deterioration). Most parents who have struggled to look after such a child are prepared to have a prophylactic abortion rather than repeat the experience. The often gloomy outlook has led some doctors to advocate non-treatment of all except mild cases, and Robert Zachary, Professor of Paediatric Surgery at Sheffield University, has recently alleged in the *British Medical Journal* that some are going further than this and practising a mild form of euthanasia. He considers, and an unknown proportion of sufferers from the condition and their parents would agree, that even a restricted life is decidedly better than no life at all.

There thus seems every reason for supporting a much wider availability of screening programmes, not just to restrict the potential for suspicions of euthanasia and not just to reduce unnecessary suffering, but also to give parents a chance to make a calmer and more rational decision at an early stage. Screening is expensive and needs staff training; it will therefore need to be provided on a selective basis, and parents will have to realise that a decision to undergo amniocentesis should not be taken unless they are prepared to go through with an induced abortion if the indications are unfavourable. Association by the Roman Catholic Archbishop of Glasgow of screening programmes with Nazi sterilisation programmes is one way of ensuring that decisions are not taken calmly. □

Benefit-cost analysis and the linear hypothesis

Alvin M. Weinberg, Director of the Institute for Energy Analysis at Oak Ridge, Tennessee warns against a common pitfall of administrators when trying to make cost/benefit analyses of new technologies

ECONOMICS is indeed the dismal science. This was brought home to me as I read the recent report (BEIR-II) of the US National Academy of Sciences, 'Considerations of Health Benefit-Cost Analysis for Activities Involving Ionising Radiation Exposure and Alternatives'. This is a sequel to the 1972 report on Biological Effects of Ionising Radiation (BEIR-I), a study that has been as widely misinterpreted and misused as any the National Academy has issued.

Benefit-cost analysis is simple in principle. In judging whether mass x-ray screening or nuclear power or therapeutic x-rays are worthwhile, one tots up the benefits—early detection of cancers, cheaper electricity, extended life span—and the costs: higher incidence of cancer or genetic disease induced by radiation. If the benefits exceed the costs, the technology is acceptable; if not it is unacceptable—QED.

Unfortunately the measures of cost (human health) and benefit (electricity) are not the same; or much worse, where the exposures are very small, the very question of estimating costs becomes essentially trans-scientific. At least at our present stage of scientific knowledge, and possibly forever, we cannot estimate the carcinogenic or genetic effect of 1 mrem of ionising radiation.

But the economists, not to say administrators, demand a neat cost/benefit calculus. This requires that a common measure, dollars per life, be used for costs and benefits; and this further requires that the vast uncertainties, even uncertainties in principle, as to the effects of low levels of ionising radiation, be obliterated by a stroke of administrative science.

That stroke of administrative scientific ingenuity is the assumption that the linear hypothesis holds down to zero dose (in other words that the biological effect is proportional to dose regardless of the size of dose or the rate of exposure), and the inevitable introduction of the unit 'person-rem'—the product of the number of individuals exposed and the dose in rems to each individual. Having brushed aside the profound illogicalities in the use of the person-rem as an estimator of actual biological damage, the economists and administrators have made the problem tractable: assign so many dollars per person-rem as the cost, so many dollars per life saved, and the cost/benefit calculus is saved.

That nonsense, of course, lies in the use of the person-rem as an estimator of damage. Curiously, the body of the BEIR-I report cautions against so using the person-rem; but the summary, possibly written by someone other than the authors of BEIR-I, in effect uses the person-rem to place bounds on the number of cancers induced by very low level exposure of large populations. BEIR-II takes up where BEIR-I left off, and unfortunately does not re-examine the validity of the original assumption concerning the use of the person-rem as an estimator of damage.

Nevertheless, in a perceptive paragraph in what I thought was the report's best chapter 'Legal and institutional aspects of using benefit-cost analysis to control ionising radiation', one reads "Serious question should be given to the adoption of alternatives to traditional economic benefit-cost analysis for such regulatory decision-making . . . One possible alternative is an appropriately comprehensive cost-effectiveness analysis . . . (that) requires the articulation of objectives, the weighing of the alternative means to achieve these objectives, and the

selection of the least costly approach". Thus, instead of performing magical balancing acts with largely indeterminate costs weighed against incommensurable and equally indeterminate benefits, concede the arbitrary, political nature of the objective at the outset—agree on an appropriately small level of radiation insult that is tolerable, and then go about devising a technology that will meet that objective as cheaply as possible.

A hint as to what the "appropriately small level of insult" should be was given in the 1958 report of the Ad Hoc Committee of the National Committee on Radiation Protection (The Friedell Report *Science*, **131**, 482; 1960). Since the biosphere has evolved in the presence of a natural background of radiation, Friedell asked whether man-made radiation ought to be judged tolerable if it were 'small' compared to this background. This very sensible suggestion was never followed up—instead, we introduced the nonsensical person-rem and strict linear hypothesis, and continued to juggle indeterminate costs and benefits.

To be sure, the definition of 'small' compared to natural background is arbitrary, but at least the arbitrariness is explicit. H. I. Adler, the former director of the Biology Division of Oak Ridge National Laboratory, has suggested (*Health Physics*, in press) that 'small' be taken as the standard deviation of the population-weighted natural background. This amounts, in the United States, to about 20 mrad per year of gamma radiation, and somewhat miraculously, is close both to the EPA emission standard for the nuclear fuel cycle and the NRC standard for emissions from nuclear power plants. The suggestion has been taken up by the American Physical Society's Committee on the Nuclear Fuel Cycle; they point out that if Adler's standard were adopted, then the actual exposures will average to something small compared to the variability in background.

Adler's suggestion amounts to cutting the Gordian knot: proving or disproving the linear hypothesis at very low exposure. The observed incidence of leukemia at Nagasaki persuades me that linearity, with slope determined by the incidence of leukemia at high dose, is inconsistent with the data. Others have looked at various bits of data and insist they see straight lines that go through the origin. But the bald fact is that linearity, upon which the apparatus of cost/benefit analysis for ionising radiation now largely rests, is, for low doses, simply an unproven, probably unprovable hypothesis. I think something like Adler's idea is a far more logical approach to standard setting.

I wax rather vehement because the use of person-rem as an estimator of damage at low dose has badly warped the nuclear debate, and it may have the same effect on the debate over the use of diagnostic x-rays. Thus most of the estimated casualties from a bad nuclear accident are found in the large dispersed population exposed to small doses of radiation. In the original Rasmussen report on reactor safety, credit was therefore given for low doses and dose rates (the strict linear hypothesis was rejected), but the critics insist on using the strict linear hypothesis. BEIR-II, despite the disclaimer previously quoted, recommends that "national policies involving activities such as nuclear power production and medical uses of radiation should be guided to the extent possible by health benefit-cost analysis"; and throughout the report strict linearity is assumed. Why could not this second BEIR report help undo the vast damage caused by the widespread misunderstanding of the first BEIR report, and state, clearly and explicitly, that the use of the person-rem as an estimator of damage is bad science, and this leads to bad policy? I believe BEIR-II must be judged a failure in not having taken the opportunity to correct this unfortunate and widespread misuse of its predecessor report. □

articles

Seafloor spreading in the western Gulf of Aden

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Interpretation of a new magnetic survey suggests that the evolution of the Gulf of Aden involved at least two phases of seafloor spreading. A recent phase from about 5 Myr ago to the present is well established and an early phase from about 30–15 Myr ago seems most likely.

A DETAILED geophysical survey was carried out in the western Gulf of Aden in 1975 aboard RRS Shackleton, mainly to discover more about the seafloor spreading history of this region¹⁻⁷. Satellite navigation was used throughout. Here we report the main results of the magnetic survey and conclude that there were at least two spreading phases. We also give a tentative location for the Arabia–Danakil–Somalia triple junction.

The survey

Sixteen profiles were obtained (Fig. 1) about 10 km apart along the direction N32°E. This direction is presumed to be the most likely spreading direction between the Somalia and Arabia plates and is computed from the best fit of the shorelines. All the seismic refraction profiles⁸ show oceanic structures. These are shown in Fig. 1 and will be used in the interpretation.

Two profiles (Fig. 2) illustrate the nature of the anomalies and their relationship to bathymetry. Note that (1) the anomalies over the axial rift and ridge contrast with those over the rest of the Gulf. The former reach more than 2,000 nT, whereas the latter typically reach only 400 nT. (2) Profile *F* shows anomalies symmetrically disposed about the axial rift valley. (3) Profile *B* shows more anomalies to the south of the axial rift than to the north.

Interpretation

Laughton *et al.*⁷ tentatively identified anomaly 5 and suggested continuous spreading over the past 10 Myr. In contrast, it has been difficult to identify anomalies over the Red Sea and establish continuous spreading beyond 3 to 4 Myr ago^{4,5,10,11}. We suggested^{12,13} at least two stages of Red Sea spreading, the first in the Oligocene, the second in the Plio–Pleistocene. As the Gulf of Aden and Red Sea are considered to result from the anticlockwise rotation of Arabia with respect to Nubia and Somalia^{3-5,14-16} and there is no evidence to a first order for shear between Nubia and Somalia, the spreading histories of the Gulf of Aden and Red Sea should be similar. The two interpretations of the magnetic anomalies are, therefore, in conflict.

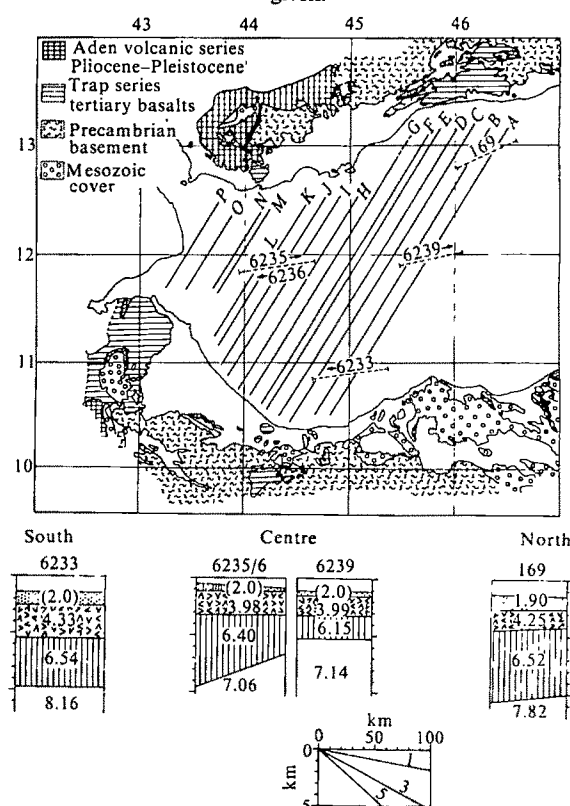
The continuous spreading model of Laughton *et al.*⁷ for the western Gulf of Aden is shown in Fig. 3. It is difficult to correlate the anomalies beyond 3–4 Myr both in number of anomalies and in their amplitudes. We therefore consider an alternative interpretation involving discontinuous rather than continuous spreading.

The correlation of the anomalies back into the Pliocene is good. At the end of the Miocene there was a spectacular change in the conditions of sedimentation in the Red Sea, probably because of some major tectonic event. Possibly, therefore, the most recent phase of spreading started at the time of the

Miocene–Pliocene boundary (about 4.9 Myr ago). Before this there seems to have been a relatively quiet period during which the main trough of the Gulf of Aden accumulated up to 1.5 km of sediment according to the seismic refraction data. For the earlier history, we may look at the geology of neighbouring Somalia and Arabia⁶. The stratigraphy of the northern and southern sides can be closely correlated up to the end of the middle Eocene. After this, sediments are confined to the littoral zones. The main trough could thus have formed between the beginning of the Upper Eocene and the end of the Miocene. The magnetic profiles were therefore compared with synthetic profiles generated for the period from 40–5 Myr.

Figure 4 shows the interpretation for the symmetrical profile *F*. A remarkably good fit can be obtained when the period 30–17 Myr is chosen for the early spreading phase. For the recent phase of spreading, a dipping layer 2 model is used in accordance with the cooling law for new oceanic crust¹⁸. For the older phase the dip is considered negligible, but a much better agreement is obtained if the magnetisation is assumed to reside in layer 3. If layer 2 is included there is too much high

Fig. 1 The locations of the sixteen total intensity magnetic profiles (A–P) in the western Gulf of Aden. The dashed lines with numbers are seismic refraction profiles⁸, the sections for which are also shown (velocities in km s⁻¹). Neighbouring geology is given.



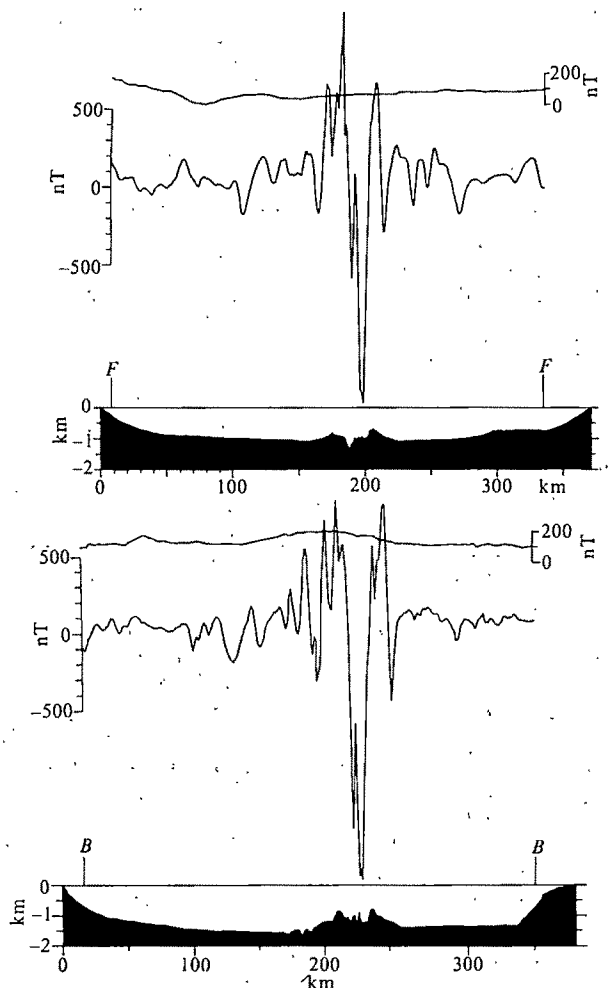
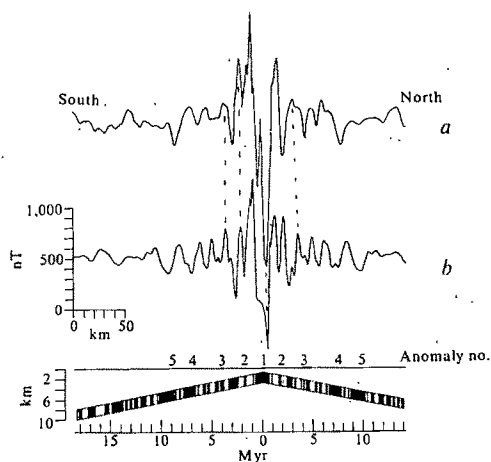


Fig. 2 Two examples of the total intensity magnetic profiles. The regional field removed was the International Geomagnetic Reference Field 1975 (ref. 9) for 1975.25 (the date of the survey) and corrections (upper profiles) for time-variations of the Earth's field were applied using base station records from Djibouti supplemented by magnetograms for H from Addis Ababa. Note the change in character of the anomalies on both sides of the axial rough zone. Vertical exaggeration 23:1.

Fig. 3 The continuous spreading model of Laughton *et al.*⁷ for the western Gulf of Aden together with (a) an observed profile (F) for comparison with (b), the synthetic anomalies, spreading rate 1 cm yr⁻¹ generated using the reversal timescale of ref. 17.



frequency signal on the synthetic profile when the depth constraints of the seismic refraction lines are used. This suggests that the magnetisation of layer 2 decays with age and layer 3 takes over in importance. This has also been noticed by Roeser¹⁹. Alternatively, the model may be too simple; for example, Blakeley²⁰ has proposed an age dependent two layer model for marine magnetic anomalies. This will be discussed further elsewhere.

For profile B (Fig. 5) there are more anomalies to the south of the axial ridge than to the north. When the contrasting amplitudes of the anomalies for the new and old phases of spreading are considered, it seems that the new phase did not start in exactly the same place as the old one stopped. The anomalies can be interpreted in harmony with the interpretation for profile F, provided the former spreading axis is some distance south of the new spreading axis. It seems that the first phase of spreading stopped about 15 Myr ago, that is at the end of the Lower Miocene.

Consideration of all the profiles shows that there are two transform faults between profiles B and F. Interpretation of B and F suggest that spreading to the west of the transform between profiles D and E stopped and started in the same place but spreading to the east of this transform restarted some distance to the north of where it stopped (Fig. 6).

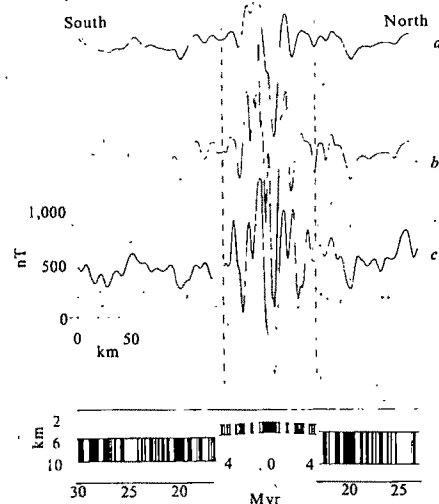


Fig. 4 Seafloor spreading model to explain the magnetic anomalies for profile F. (b) Shows the observed anomalies and (a) the upward continued anomalies (1.83 km). The latter is helpful in identifying various marker features. (c) Shows synthetic anomalies considered to give the best fit. Spreading rates: centre 1 cm yr⁻¹; S limb 1 cm yr⁻¹; N limb 1.13 cm yr⁻¹. These have been computed using the usual simplifying assumption of vertically sided blocks. The seismic refraction profiles (Fig. 1) have been used for the depths and thicknesses of the magnetised layers.

Discussion

The evidence suggests the following history of the Gulf of Aden. First, major movements probably occurred 40 Myr ago; by the end of the Upper Eocene the Gulf of Aden downwarp was in existence and the first major phase of seafloor spreading began 30 Myr ago (beginning of Upper Oligocene). This phase continued until about 15 Myr ago (end of Lower Miocene). There was then a quiet period during which up to 1.5 km of sediment accumulated. At the end of the Miocene, spreading recommenced continuing to the present. We emphasise that this interpretation is the simplest which fits the observations; the possibility of more than two spreading phases cannot be excluded.

The dates for the early stage of seafloor spreading (30–15 Myr) agree well with the dates of basaltic activity in neighbouring regions²¹. They differ from the dates (41–34 Myr) deduced for the Red Sea¹². At that time, the choice of model was constrained by the dates (36.4 ± 2, 36.7 ± 2, 30 Myr) for rocks in

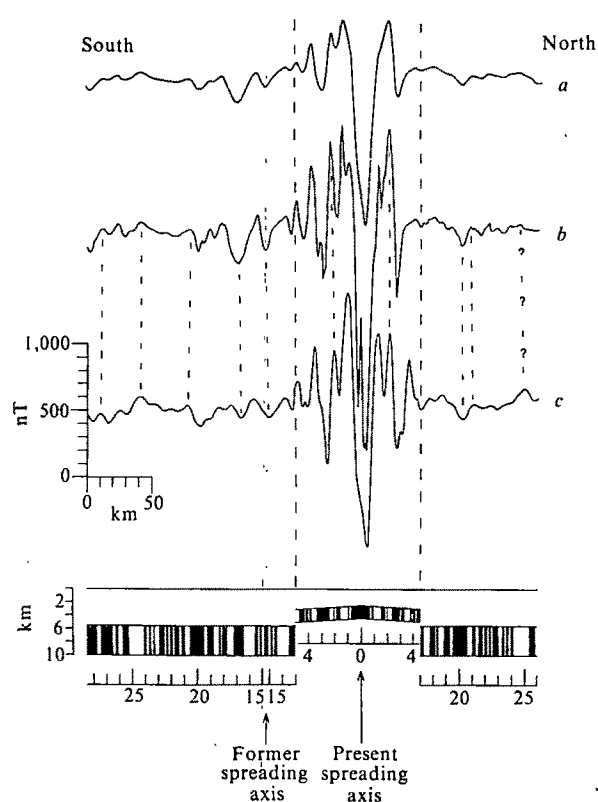


Fig. 5 Interpretation of profile B. Note the smaller amplitude of the observed anomalies on the northern side due to the proximity of a transform fault. (a) Shows the anomalies upward continued to 1.83 km, (b), observed anomalies at sealevel and (c), synthetic anomalies with spreading rate 1 cm yr^{-1} .

Red Sea boreholes²². These are now known to be in error (Nelson, personal communication) and a re-examination of the Red Sea magnetic data (to be published) shows that the magnetic anomalies for the Red Sea can be interpreted in harmony with the seafloor spreading history of the western Gulf of Aden.

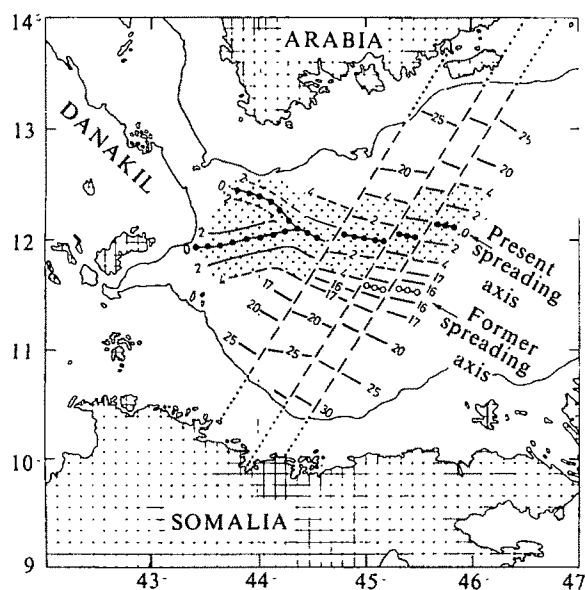


Fig. 6 Isochrons (Myr) deduced from magnetic anomalies for the western Gulf of Aden. The present spreading axis (zero isochron) is shown by a chain of solid circles and the former spreading axis (~ 15 Myr isochron) is shown by a chain of open circles. The three transforms are shown by dotted lines. Land over 3,000 feet (914 m) is cross-hatched.

This has several features in common with an early interpretation by Laughton² who suggested that the development of the Gulf of Aden was a consequence of two distinct phases of continental drift. In phase 1, the continental crust was envisaged to be stretched, thinned and intruded by basaltic dykes. After this there was a period of 'reduced movement' during which the main trough accumulated 0.5 to 1.5 km of sediment. This was followed by phase 2 in which new oceanic crust formed the central rough zone. The new interpretation replaces Laughton's phase 1 by a major period of seafloor spreading over a period of some 15 Myr through the Upper Oligocene and Lower Miocene.

Plate geometry

Figure 6 summarises the magnetic interpretations. From most of the magnetic profiles, it is possible to identify the positions of the 2 and 4 Myr isochrons and these are shown. The region between the 0 and 4 Myr isochrons is stippled, illustrating the minimum extent of the new phase of spreading. The strike of the spreading axis is $N110^\circ E$. Previously⁷, the transforms and offsets had not been recognised and the spreading axis was considered to be continuous with a strike of $N80^\circ E$. The new interpretation is consistent with the ridge-transform pattern further east⁷ and with the direction of separation determined from the fit of the coastlines^{15,16}. In the west, the present spreading axis bifurcates, one branch going towards the Straits of Bab-el-Mandeb and the other towards the Gulf of Tadjura. The bifurcation marks the present location of the triple junction for the Somalia-Arabia-Danakil plates.

For the older spreading phase, the 16, 17, 20 and 25 Myr isochrons are shown. In the extreme south, it is possible to identify tentatively the 30 Myr isochron (close to the Somali shore). This implies that the minimum separation between the Somalia and Arabia plates is 380 km and that a considerable proportion of the Arabia and Somalia coastal plains to the west of $45^\circ E$ must be underlain by 20–30-Myr-old oceanic crust.

In the east of the survey area, the new spreading axis is about 75 km north of the old spreading axis. The transforms associated with the old and new spreading phases are approximately collinear and the isochrons are all approximately parallel. This implies that within the limits of accuracy of navigation, the poles of rotation for the two phases of movement must be very similar and close to the pole of rotation for the total movement, that is $26.5^\circ N$, $21.5^\circ E$ ^{14,16,23}.

We thank Captain P. Warner and the officers and crew of the RRS Shackleton, also Dr J. C. Lepine (Djibouti) and P. Gouin (Addis Ababa) for help with base station magnetometer records; and A. S. Laughton and R. B. Whitmarsh (I.O.S. Wormley) for interest and encouragement. The work was financed by Natural Environment Research Council Grant GR/3/2467.

Received 26 May; accepted 14 November 1977.

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Structural evidence for gene duplication in the evolution of the acid proteases

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X-ray studies of acid proteases indicate a bilobal structure with a well defined active site cleft. An intramolecular two-fold symmetry axis relates two topologically similar domains and the active site residues. A possible mechanism for evolution by gene duplication, divergence and gene fusion is presented.

X-RAY crystallographic studies have shown that proteins are organised into globular domains. Those proteins of molecular weights <25,000 have domains comprising between 100 and 200 amino acid residues. Generally the amino acids in one domain fold individually and are then organised together to give the native tertiary structure¹. Further it is possible that the proteins evolved from smaller functional units by gene fusion; thus lactate, alcohol and glyceraldehyde phosphate dehydrogenases have nucleotide binding domains strongly resembling each other linked to quite different domains which contribute towards substrate specificity^{2,3}. A more primitive nucleotide binding protein may have been a common ancestor³. In other proteins where gene duplication had occurred, it may have been selectively advantageous to follow this by gene fusion leading to a protein with two similar globular domains. We present here evidence for such a mechanism in the evolution of acid proteases leading to a bilobal structure in which an approximate twofold symmetry axis relates two topologically similar domains and the active site aspartate residues.

Acid proteases are so called because they have an optimal catalytic activity at acid pH (for a general review, see ref. 4.) They include mammalian digestive enzymes such as pepsin, chymosin and cathepsin D, microbial proteases such as those produced by *Penicillium janthinellum* (penicillopepsin)^{5,6}, *Rhizopus chinensis* (rhizopuspepsin)⁷, and *Endothia parasitica* (endothiapepsin)^{7,8} and probably certain enzymes involved in mammalian control processes, for example renin, which acts on angiotensinogen.

These acid proteases are characterised by two active site aspartates and most are inhibited by the microbial peptide analogue, pepstatin. They tend to cleave between hydrophobic amino acids but secondary interactions are important in determining specificity⁹. The sequence of porcine pepsin¹⁰ shows that the enzyme has 327 amino acids with the active site aspartates at residues 32 and 215 (see Fig. 1). Pepsin, penicillopepsin⁶ (Fig. 1) and chymosin¹¹ are closely homologous structures and fragments of the sequences of other microbial enzymes¹¹ strongly indicate an homologous series. Medium resolution X-ray analyses of rhizopuspepsin⁷, endothiapepsin^{7,8} and penicillopepsin^{5,6} confirm this structural homology. Preliminary comparisons of the three structures show root mean square deviations between topologically equivalent α carbons^{12,13} (about 200 of each enzyme) of the order of 1.35 Å (data not shown). A stereo drawing of the α -carbon positions of one of these, penicillopepsin, is shown in Fig. 2. The numbering of the

residues is chosen to facilitate comparison with the sequence of pepsin and is given in Fig. 1. The bilobal structure defines an extensive cleft in which the active site residues Asp 32 and Asp 215 are positioned. The two lobes comprise residues -4 to 171, and 177 to 327, connected by a short length of peptide, 172-176. The secondary structure is predominantly β sheet and the general folding is illustrated schematically in Fig. 3. The identity of the residues in the strands is indicated in Fig. 1, and this nomenclature is derived from that described by Hsu *et al.*⁶. It should be noted that Fig. 3 does not indicate the lengths of the β -sheet strands nor of the connecting loops. Furthermore, the extensive sheet is sharply twisted at several points, for example at strands c and m, and this is more clearly illustrated in Fig. 4. The sequences of pepsin and chymosin strongly suggest that they also have similar secondary and tertiary structures.

Internal structural homology

Tang *et al.*¹⁴ noted the remarkable similarity between the residues 30-42 and 213-225 (see Fig. 1) of pepsin. These two stretches of polypeptide include the two active site aspartyl residues at positions 32 and 215. They suggested that this may have been a consequence of gene duplication and fusion, but the pepsin sequence gave no further indications to support this hypothesis. However, consideration of the secondary structure now shows that these residues form similar β structures (c_2 -d and m_2 -n in Fig. 4) and in each case are crossed by a strand (h_1 parallel to c_2 and p_1 parallel to m_2) which gives rise to a psi (ψ) shaped structure. These parallel strands h_1 and p_1 (see Fig. 1) also have some sequence homology and in each case are about 90 residues towards the C terminus from the active site aspartates. In fact, the similarity is considerably more extensive than this as can be seen from Fig. 3. A twofold symmetry axis positioned between antiparallel strands i and q relates the structure in the N-terminal lobe to that in the C-terminal lobe. In particular the active site aspartates, the parallel pairs of strands in the otherwise antiparallel β sheet, the small sections of helix (h_2 and p_2) and the interstrand connectivities in the lobes are equivalent. This striking symmetry is carried over to a large extent into the tertiary structure, as can be seen from Figs 2 and 4. However, the tryptophan residues thought to be equivalent on the basis of the primary structure¹⁴ are not in symmetry related positions.

In order to quantify this symmetry relationship we have compared the two lobes using a least-squares refinement programme for 'topologically equivalent' residues^{12,13}. This evaluates the symmetry in runs of contiguous sequence using a progression rule. In penicillopepsin 61 residues of each lobe are topologically equivalent, and the root mean square deviation for these is 2.01 Å. The two lobes are shown superposed in Fig. 5. The rotation angle is close to that of a twofold but not exact; it is 174.6° and there is a translation of 1.0 Å along this rotation axis relating the two lobes.

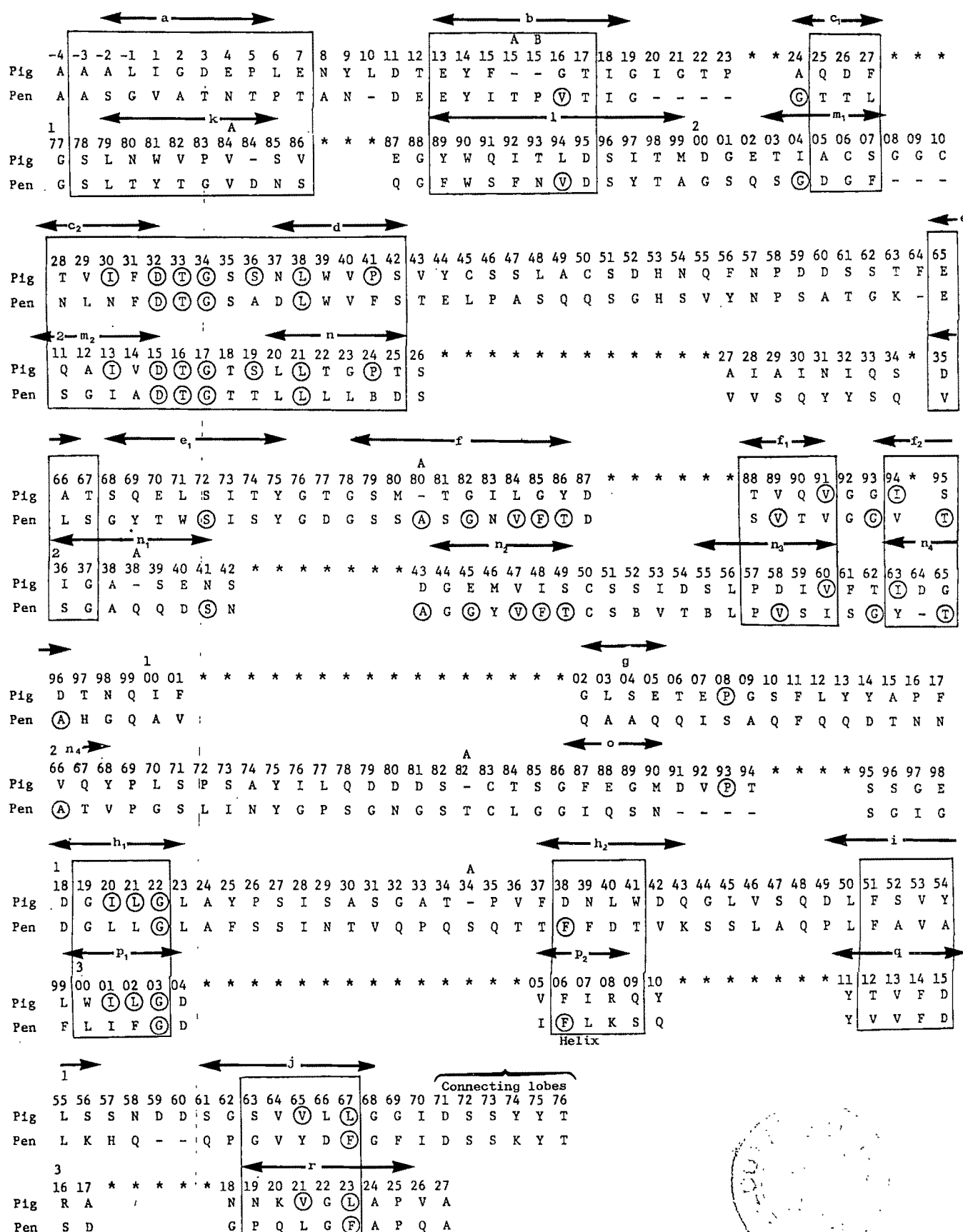


Fig. 1 The sequences of the acid proteases, porcine pepsin and penicillopepsin, aligned to maximise the internal structural homology. Residues in boxes are 'topologically equivalent residues'. Circles indicate residues which are identical in the two lobes. Asterisks identify positions where there is no topologically equivalent residue. The residue numbering is that of porcine pepsin and the strands are lettered to facilitate discussion in the text and identification in the schematic Figs 3 and 4.



Fig. 2 Stereo drawing of the α -carbon positions of the acid protease, penicillopepsin. The residue numbering given in Fig. 1 is designed to facilitate comparison with the homologous pepsin structure. Structures of the acid proteases, endotheiapepsin and rhizopuspepsin, are very closely related. The enzyme is viewed along the approximate twofold symmetry axis relating the two lobes of the enzyme as described in the text.

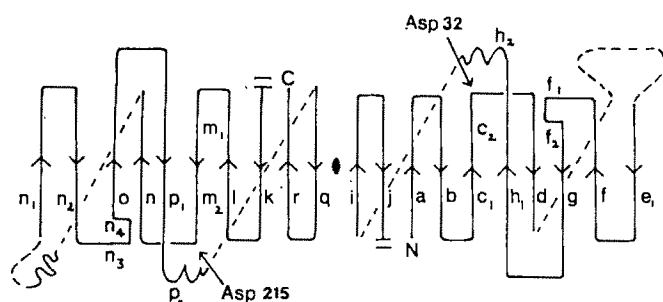
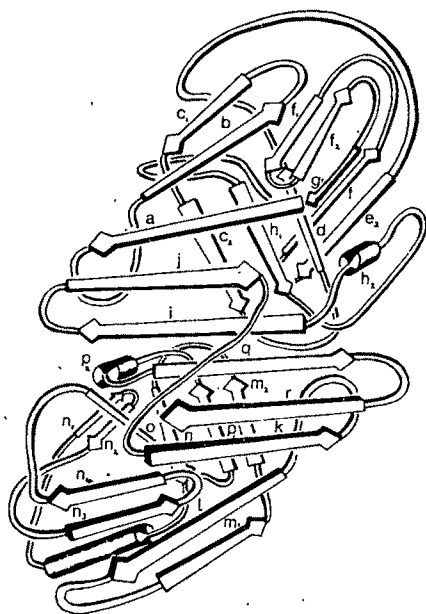


Fig. 3 A schematic drawing of the secondary structure found in the acid proteases. The strand labels are defined in Fig. 1. Strands a-j are in the N-terminal of Fig. 2 and related by an approximate twofold symmetry axis to the strand k-r in the C-terminal lobe. There are rather large angles between some strands such as d and g, n and o; strands c and m bend in the centre as shown in Fig. 4.

Fig. 4 A schematic drawing of the tertiary structure of the acid protease, endotheiapepsin, viewed along the twofold axis. The strand labels are defined in Fig. 1.



The 61 topologically equivalent residues are indicated in Fig. 1 by inclusion in boxes. Assuming porcine pepsin is homologous there are 58 such residues in this enzyme. The sequences are aligned to optimise the topological equivalence. This inevitably leads to gaps which are indicated by asterisks in Fig. 1. These correspond to loops of polypeptide which are present in one lobe but absent in the other.

Several of the differences between the lobes correspond to extensions or deletions of β loops connecting two adjacent antiparallel strands of the β sheet. Thus the loop k-l is a few residues shorter than a-b, and b-c₁ is much less extended than l-m₁, and q-r is less extended than i-j. A more striking example is the loop n₁-n₂ which is seven residues shorter than e₁-f. In other places, for example d-e compared to n-n₁, the chain folds across several strands of sheet and a larger length of less tightly folded polypeptide is inserted on one side (residues 43-55 between d and e). Similar additions and deletions occur in the strands connecting h₁-h₂-i and p₁-p₂-q; in this example rather long stretches of polypeptide are inserted between h₁-h₂ and h₂-i compared to p₁-p₂ and p₂-q, but the two helices h₂ and p₂ nevertheless partially overlap (see Fig. 1).

The insertions which give rise to difference between the lobes are on the surface or available to the surface of the enzyme and do not interfere with the general tertiary fold. Indeed, deletions or additions occur often between penicillopepsin and pepsin in positions which are topologically inequivalent between the lobes; for example the deletion of residues 20-23 (between b and c₁) of pepsin in penicillopepsin. Further, the loop e₁-f which differs from n₁-n₂, is completely on the surface, and these residues in penicillopepsin differ from the equivalent loop in endotheiapepsin and rhizopuspepsin.

Evolution by gene duplication

The two lobes of the acid proteases thus have approximately 35% of the residues in topologically equivalent positions. These results are similar to those observed for the bacterial and pancreatic serine proteases with an important difference. It was first pointed out by Shotton and Watson¹⁵ that in elastase the two domains have a similar fold, but the twofold relationship is such that the polypeptide chain of the C-terminal domain has the opposite polarity to that in the N-terminal domain. This has subsequently been observed in the bacterial serine proteases where the twofold relationship between domains is more exact^{16,17}. It can be concluded, therefore, that the similarity of conformation in the two domains of the serine proteases is a

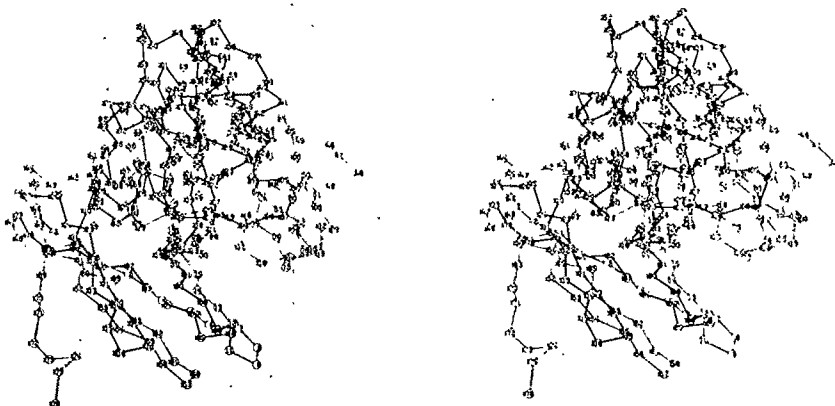
result of specific structural requirements rather than a gene duplication event. This may not be the case for the acid proteases for the twofold relationship between the two lobes leaves the chains with the same polarity. However, the lack of sequence homology is striking. Of the 61 topologically equivalent residues there are only 10 identical residues in penicillopepsin and 14 in porcine pepsin. This may be taken to support the view that the acid protease fold has evolved convergently in the two lobes. Alternatively it indicates that the secondary and tertiary structure is conserved in evolution to a greater extent than the primary structure of a protein.

The acid protease fold described here involves the stacking of β sheets together. If divergent evolution has occurred in the two lobes, complementary changes must have occurred in residues

fusion would be selectively advantageous in assuring the correct association of dissimilar subunits. These steps are shown schematically in Fig. 6. In fact the connecting peptide between subunits is in a surface position and probably could be cleaved without denaturing the enzyme. Indeed, Rajagopalan *et al.*²⁵ have shown that porcine pepsin carrying various cleavage sites is still catalytically active and it is fascinating that this may be a reflection of the evolution of the enzyme from two smaller globular proteins.

This outline for the evolution of the acid protease structure is supported by analogous observations made in several other protein structures. Crystallographic studies of the ferredoxin from *Clostridium pasteurianum* show that the iron and labile sulphur atoms are arranged in two clusters each of which is

Fig. 5 A stereo drawing of the α carbons of the N-terminal lobe (open virtual bonds) superposed on the α carbons of the C-terminal lobe (closed virtual bonds) after rotation by the approximate twofold symmetry operation described in the text.



contributing to the core between these sheets, possibly in a protein formed by gene duplication and not under selective pressure¹⁸. Although changes may occur in one of a complementary pair of amino acid side groups which give a poorly folded or unfoldable structure, the same fold may later be optimised by further changes.

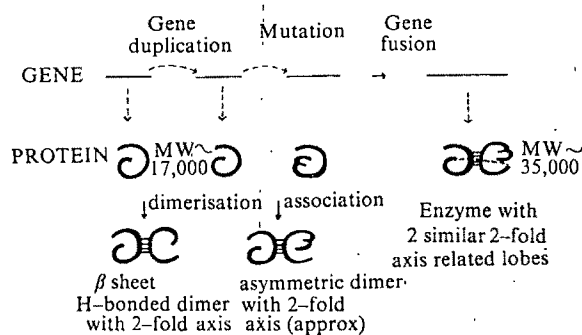
Thus it seems possible that acid proteases evolved by gene duplication of an ancestral protein of about 150 residues having a fold similar to that of one lobe of pepsin. Even before duplication a dimer of two identical subunits may have existed. The extensive hydrophobic interactions and the antiparallel pleated sheet between the two lobes of the acid protease (strands q and i) are reminiscent of similar quaternary interactions involving antiparallel sheet in insulin¹⁹ and liver alcohol dehydrogenase dimers²⁰, and in concanavalin^{21,22} and prealbumin tetramers²³. After the gene duplication event the two subunits evolved divergently so that the carboxyl groups of the two aspartyl residues developed different *pK*s and a mechanism of hydrolytic cleavage, as described recently²⁴. Finally, gene

enclosed by a similar folding of the polypeptide chain²⁶. In the bacterial ferredoxins, however, the sequences of the two similar folding units are more homologous than that which we report here for the acid protease folding domains. Rhodanese, the sulphur-transferring enzyme from bovine liver, consists of a single polypeptide chain which folds into two nearly identical domains, but only 14 of the 109 topologically equivalent residue pairs are identical in sequence²⁷. This latter figure is equivalent to the lack of homology between the two domains of the acid proteases, and indicates that a homologous amino acid sequence is not a necessary condition for similar tertiary structures. This phenomenon of folding domains that are similar in conformation but with widely differing primary structure would seem to be a rather common one.

We thank Mrs E. Wood for drawing Figs 3 and 4. J.A.J. and T.L.B. thank the SRC for support.

Received 3 October; accepted 16 December 1977.

Fig. 6 A schematic representation of the possible evolution of the acid proteases by gene duplication.



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Purification of mouse interferon by sequential affinity chromatography on poly(U)- and antibody-agarose columns

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Mouse interferon has been purified to homogeneity by two-step affinity chromatography. Two polypeptide bands were obtained on sodium dodecyl sulphate-polyacrylamide gel electrophoresis migrating at molecular weights 35,000 and 22,000, both having antiviral activity. The 35,000 but not the 22,000 band, also stained with periodic acid-Schiff. The specific activity was 8×10^6 of our laboratory units, corresponding to 2.4×10^6 NIH reference units.

COMPLETE purification of interferon will make it possible to determine whether the varied biological effects attributable to interferon preparations are due to the interferon itself. Furthermore, analysis of the structure of interferon may reveal its active site(s) and eventually lead to its synthesis *in vitro*; also by labelling the molecule, it will be possible to investigate the initial interaction of interferon with the cell. Although techniques have been developed for the large-scale production¹ and purification of mouse interferon by affinity chromatography²⁻⁷, total purification has not been achieved. Knight, using gel filtration and ion-exchange chromatography, reported that purified mouse L cell interferon still had "10 to 11 polypeptide" bands after sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE)⁸. We now report the purification of mouse C-243 cell interferon with a high final recovery of

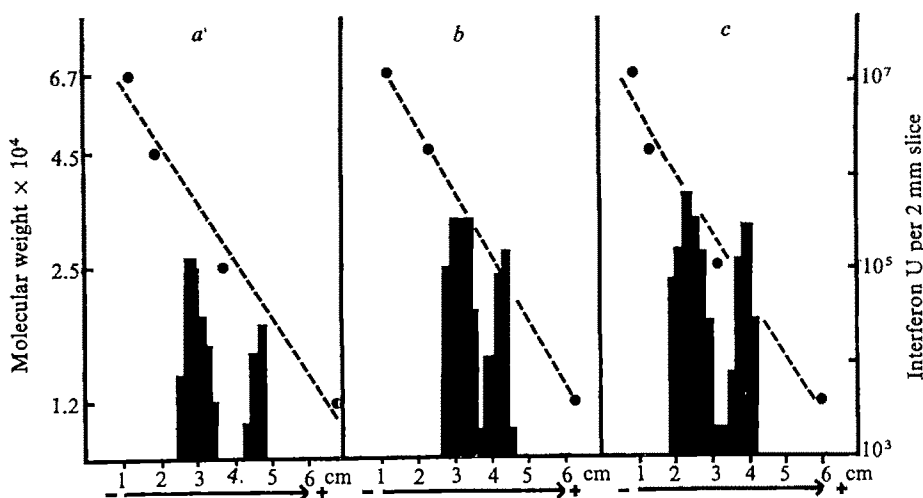
biologically active and pure material by SDS-PAGE.

Interferon titrations were performed using either a microtitre assay in L cells or a plaque reduction assay in 5-d-old secondary cultures of mouse embryo fibroblasts (MEF), with vesicular stomatitis virus (Indiana strain) as challenge virus. One microtitre unit per 0.2 ml corresponds to 10 MEF units per ml. All titres are expressed in MEF units. One MEF unit equals 0.3 NIH reference units.

Electrophoresis on acrylamide slab gels

Either plain 13% or 15% gels or exponential 10–20% gradient gels were prepared in slabs 0.075 cm thick, 11 cm long and 16 cm wide. A 5% stacking gel 1 cm high was prepared with sample wells of either 0.025 or 0.05 ml. The discontinuous buffer system using SDS has been described by Laemmli⁹. Before electrophoresis, samples were dialysed for 18 h at room temperature against 0.125 M Tris-HCl, pH 6.8, 1% SDS and 10% glycerol. β -Mercaptoethanol (1%) was added sometimes. After dialysis, some samples were concentrated 10- or 15-fold with a Minicon-B concentrator, with 15,000 molecular weight cutoff (Amicon, Lexington). Gels were run for 5–7 h depending on the concentration of the acrylamide at a constant current of 10 mA per gel, and 20 μ l of sample was usually applied per slot. After electrophoresis, gels were stained for 15 min in a solution of 0.1% Coomassie brilliant blue in a mixture

Fig. 1 Electrophoretic profile of interferon activity recovered from 10–20% acrylamide gradient gels. The starting material for (a) was 20 μ l crude interferon, concentrated tenfold, and containing 2.6×10^6 U; for (b) it was 20 μ l of interferon purified on an immunosorbent column (Fig. 2) and containing 3×10^6 U; and (c) it was 20 μ l of interferon purified on poly (U)-Sepharose and containing 5×10^6 U. Crude interferon was used as starting material both for the chromatography on anti-interferon globulin bound to Affigel-10 and on poly (U)-Sepharose. ---●---, Migration of molecular weight markers. For the elution of interferon from acrylamide gels, they were fractionated into 2-mm slices. Optimal results were obtained consistently when slices were eluted for at least 18 h at 4°C in electrode buffer (0.025 M Tris, 0.2 M glycine and 0.1% SDS) using 0.2 ml per slice. Total recovery of interferon activity was obtained in these conditions. The form of the gel changed as a result of staining and drying and it was not possible to correlate precisely the antiviral activity eluted from the slices obtained from a fresh gel with the stained bands of a dried preparation. This difficulty was circumvented as follows. Two aliquots of the same gel were deposited into each of two slots, with an empty slot in between. At the end of the run, before staining, the gel was cut longitudinally along a line centred on the empty slot, and care was taken not to displace the two parts of the gel with respect to each other. Another longitudinal cut, parallel to the first, was made at the other side of the sample run to be eluted; this gave a slice 1 cm wide. The latter was cut transversely every 2 mm, and each cut was prolonged a few millimetres every 2 mm, and each cut was prolonged a few millimetres into the gel reserved for staining. It was thus possible, after staining and drying, to correlate the antiviral activity of each fraction with protein bands by counting the notches.



of 50% methanol, 10% acetic acid and 40% distilled water; they were destained overnight in a solution of 7% acetic acid and 5% methanol in distilled water and then dried.

Crude interferon preparation

Mouse interferon was prepared from suspension cultures of Swiss mouse C-243 cells induced with Newcastle disease virus (NDV) and titred 1.3×10^8 U ml⁻¹; its protein content was 0.1 mg ml⁻¹ and its specific activity was 1.3×10^7 U per mg of protein.

The crude starting interferon preparation, when electrophoresed in the presence of SDS in non-reducing conditions, contained two peaks of activity, one migrating at 35,000 molecular weight (MW), representing about 80% of total activity, the other migrating at 22,000 MW and representing about 15% of the activity (Fig. 1a). These findings are in good agreement with those reported by Stewart *et al.* for NDV-induced L-929¹⁰ and C-243 cell¹¹ interferons (for the latter interferon they reported a molecular weight of 38,000 rather than 35,000). Likewise, we have found that treatment of interferon in reducing conditions (buffer supplemented with 1% β -mercaptoethanol) resulted in a 90% or more reduction of the antiviral activity of the 22,000 MW peak, whereas the activity of the 35,000 MW fraction was unaffected.

Interferon obtained from poly(U)-agarose and antibody-agarose

Affinity chromatography of C-243 cell interferon on poly(U)-Sephacrose 4B (Pharmacia) has been described before¹². Briefly, an interferon sample, predialysed for 24 h in 10 mM Tris-HCl, pH 7.5, is applied to the gel and desorption is obtained by adding 1 M NaCl to the Tris buffer. By this simple procedure,

we obtained mouse interferon preparations with specific activities of up to 10^8 U per mg of protein. Furthermore, recovery was total with both peaks of activity (35,000 and

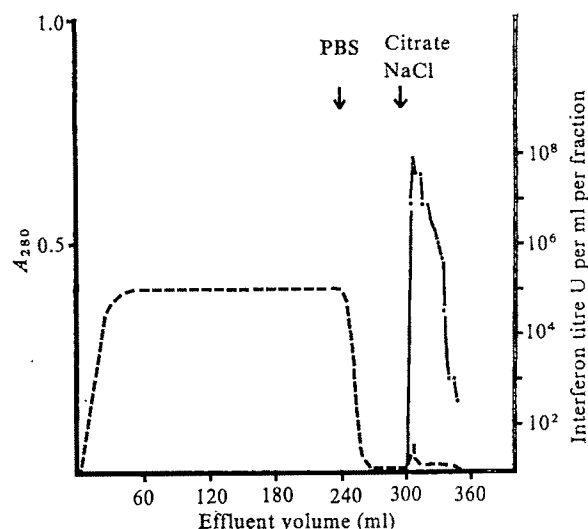


Fig. 2 Affinity chromatography of C-243 cell interferon on an anti-interferon globulin Affigel column. Anti-interferon serum was prepared and partially purified as before¹³. The partially purified anti-mouse interferon serum used had a neutralising titre of 2.4×10^6 U when assayed against 16 U of interferon. One ml of serum neutralised 6.4×10^8 interferon reference units. Immunoglobulins were precipitated with 2.1 M ammonium sulphate, processed as before² and coupled to an agarose matrix with a spacer arm, Affigel-10 (Bio-Rad). The globulin fraction obtained from 15 ml of the anti-interferon serum was fixed to 1 g of Affigel-10. A crude interferon preparation (240 ml) containing a total of 3.12×10^8 U was applied to the column. The loaded column was washed with 10 bed volumes of phosphate-buffered saline (60 ml) and desorbed with citrate buffer (1 M NaCl, 0.1 M Na citrate, pH 2.2); 3.0-ml fractions were collected. Total recovery was 3.7×10^8 U, that is 119% of input, and the specific activity of the pooled peak fractions was 2×10^8 U per mg of protein. An aliquot of the peak fraction with the highest titre was dialysed against sample buffer, concentrated 10-fold, and analysed on a 15% acrylamide gel (Fig. 3a, channel 3). Protein content was determined by the fluorometric assay described by Boehlen *et al.*¹⁴. In some instances the protein content was estimated after PAGE from the intensity of the staining of the bands with Coomassie brilliant blue. This was done by comparison with bands obtained after electrophoresis of known amounts of bovine serum albumin and ovalbumin. — — —, Absorbance at 260 nm; —●—, interferon titre.

Table 1 Summary of two-step purification procedure

	Volume (ml)	Titre (U ml ⁻¹)	Total U	Specific activity (U per mg of protein)
Starting material	200	1.3×10^6	2.6×10^8	1.3×10^7
Peak fractions from poly(U)-Sephacrose column	(1) 5 (2) 5 (3) 5 (4) 5	1.0×10^7 4.0×10^7 1.0×10^7 1.3×10^6	3.0×10^8 (115% of starting material)	1.2×10^8 (pool 1-4)
Peak fractions from solid phase immuno-adsorbent column	(1) 2.5 (2) 2.5 (3) 2.5 (4) 2.5 (5) 2.5	4.0×10^7 4.0×10^7 2.0×10^7 2.5×10^8 1.3×10^6	1.0×10^8 * (38% of starting material) 1.5×10^8 † (52% of starting material)	ND 8.0×10^8 8.0×10^8 ND ND

Crude starting material (200 ml) was dialysed overnight against 10 mM Tris-HCl buffer, pH 7.5, and was applied to a poly(U)-Sephacrose column (total bed volume 10 ml). The column was then rinsed with 10 bed volumes of Tris-HCl and desorption was carried out by adding 1 M NaCl to the buffer. The four 5-ml desorption fractions were pooled and diluted fivefold in 10 mM Tris to decrease the molarity of the preparation to be applied to the solid phase immuno-adsorbent. Thus 100 ml was applied to the anti-interferon globulin-agarose column (bed volume 4 ml). After absorption, the column was rinsed with 10 bed volumes of phosphate-buffered saline and desorption was carried out in 0.1 M citrate buffer, pH 2.2, supplemented with 1 M NaCl. Fractions (2.5 ml) were collected and the bulk of interferon activity was recovered in fractions 1-3. These fractions were concentrated 15-fold and analysed electrophoretically. Fraction 1 still contained some contaminating polypeptide bands in the low molecular weight range (<20,000) but fractions 2 and 3 showed only the two bands corresponding to interferon activity.

*This material was still slightly contaminated with other polypeptides.

†This material was electrophoretically pure. (Fig. 3a, channel 4.)

22,000 MW) being recovered (Fig. 1c). As Fig. 3a shows (channel 2), the two bands corresponding to interferon can be seen after staining. In addition to the two polypeptide bands corresponding to interferon, there are many other contaminating polypeptides.

Purification of C-243 cell interferon on antibodies covalently bound to agarose through a spacer arm (Affigel-10) is described in Fig. 2 and the product is illustrated in Fig. 3. Interferon recovery was complete and, on electrophoresis a migration pattern comparable with that of the starting material was obtained (Fig. 1b). Again, although an extensive degree of purification was achieved (230-fold to yield a product with specific activity about 3×10^8 U per mg of protein), contaminating polypeptides were still present in the preparation (Fig. 3a, channel 3). Because the polypeptide contaminants retained by the affinity chromatography of the crude interferon preparation on poly(U)-agarose were different from those retained by the antibody-agarose column, we decided to combine the two techniques.

Sequential affinity chromatography

The crude interferon preparation was first purified on poly (U)-Sephacrose and the product was applied to an anti-interferon globulin column. Experimental details and results are given in Table 1 and illustrated in Fig. 3a, channel 4. After concentration and electrophoresis of an aliquot of fractions 2 or 3,

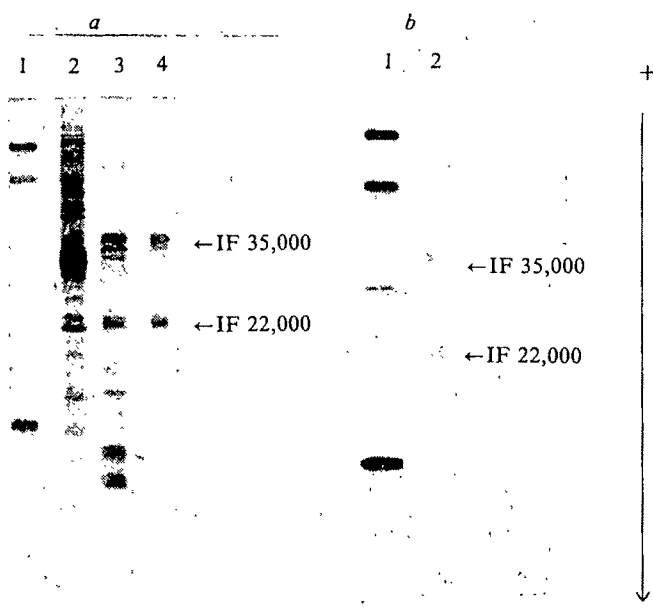


Fig. 3 SDS-PAGE in a 15% gel of interferon (IF) preparations of different degrees of purity. *a*, Input per channel was 20 μ l in each case. Channel 1, molecular weight markers; channel 2, after purification on poly (U)-agarose; input was 1×10^7 U; channel 3, after purification on antibody-agarose; input was 1.3×10^7 U; channel 4, after combination of the two methods; input was 1.2×10^7 U. *b*, Migration of interferon after boiling in denaturing conditions. Channel 1, molecular weight markers; channel 2, purified preparation containing 6×10^6 U in 10 μ l. Molecular weight markers from top to bottom: bovine serum albumin, ovalbumin, chymotrypsinogen and cytochrome *c*.

only two protein bands migrating at 35,000 and 22,000 MW were observed. When slices of the uncoloured gel corresponding to these two bands were eluted for biological activity, there was excellent correlation between interferon activity and the stained bands (Fig. 4). As Fig. 3a, channel 4, and Fig. 3b, channel 2 show, this preparation can be considered pure because no other bands were detected (the limits of detection with the method in our hands was 0.07 μ g of either bovine serum albumin (BSA) or ovalbumin). In one experiment we put 2.5 times as much material on the gel as was used for Fig. 3a, channel 4, by starting the electrophoresis with 20 μ l and refeeding twice, each time with 15 μ l (using a higher stacking gel). The two interferon bands were more diffuse than those obtained in normal conditions, which was expected in view of the way the material was placed on the gel, but again no contaminating bands were observed. In addition to electrophoresis on a 15% gel, the pure material was run in a 13% gel and in a 10–20% gradient gel, and each time antiviral activity was present in gel slices corresponding to the two stained bands.

For the interferon band to be quite distinct, it was necessary to put a minimum of 20 μ l per slot of a preparation of titre 3×10^8 U ml⁻¹ (that is 6×10^6 U per slot). In these conditions the intensity of the stain with Coomassie brilliant blue corresponded roughly to 0.75 μ g of protein, for the two bands combined, using BSA as standard. This represents 37.5 μ g of

interferon per ml for a preparation of titre 3×10^8 U ml⁻¹, which means that the specific activity was 8×10^6 U per mg of protein. The 35,000 MW band was rather broad, but such dispersion is not unusual for glycoproteins and there is evidence that at least some interferons are glycoproteins^{15,16}.

To determine whether the purified product contained sugar we stained the gel with periodic acid-Schiff (PAS) after PAGE in a 15% gel. We used 15 μ l of the same concentrated preparation used to stain the polypeptide bands with Coomassie brilliant blue as shown in Fig. 3. While the 35,000 band stained almost as intensely with Schiff's reagent as with Coomassie brilliant blue, the 22,000 band hardly stained at all. No other bands were visible. Thus there was more sugar present in the 35,000 MW fraction than in the 22,000 MW fraction.

To exclude the possibility that the 35,000 MW component was a dimer of the 22,000 MW component, an aliquot of the preparation that had been electrophoresed for activity (Fig. 3a, channel 4 and Fig. 4) was supplemented with 1% β -mercaptoethanol and boiled for 1.5 min before electrophoresis. This treatment did not affect migration of either band (Fig. 3b, channel 2).

Correlation between antiviral and cell multiplication inhibitory activities

Individual fractions, after SDS-PAGE, of the 35,000 band purified interferon were tested for antiviral activity on L cells

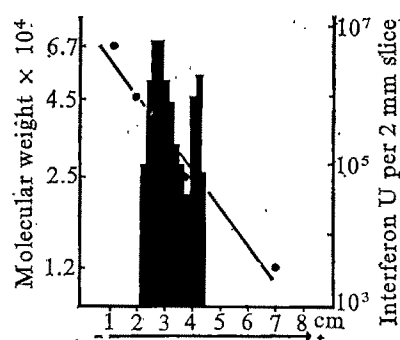


Fig. 4 Profile of interferon activity recovered after electrophoresis of the pure preparation. An aliquot of fraction 2 obtained after desorption of the anti-interferon globulin-agarose column (Table 1) was dialysed and concentrated 15-fold before electrophoresis (see legend to Fig. 1). A 20- μ l sample was electrophoresed in a 15% acrylamide gel for staining (Fig. 3), and in an adjacent channel another 20- μ l sample was electrophoresed to recover activity. The theoretical input was 1.2×10^7 U per channel. The total recovery of interferon activity after electrophoresis was 1.9×10^7 U (158% of input); 79% of this activity was recovered from the three slices corresponding to the 6-mm area of the stained 35,000 molecular weight band, and 19% from the two slices corresponding to the 4-mm area of the 22,000 band. The remaining 6% was recovered from the area in between and adjacent to the bands. Comparable results were obtained when the electrophoresis was carried out either in a 13% or in a 10–20% acrylamide gradient gel. —●—, Migration of molecular weight markers.

and cell multiplication inhibitory activity on murine leukaemia L1210 cells, as described before^{11,17}. As Fig. 5 shows, there was an excellent quantitative correlation between the two activities.

We conclude therefore that mouse interferon can be purified considerably by affinity chromatography using poly (U)-Sephacrose¹² or anti-interferon globulin columns². Combining both techniques, we obtained interferon that seemed pure after SDS-PAGE—we consistently obtained only two bands after staining either with Coomassie brilliant blue or PAS.

The fact that both the 35,000 and 22,000 MW components were purified together on the antibody-agarose column does not imply that they are antigenically identical, for immunisation had been carried out with the crude starting material containing both components. They were also purified together on the poly (U)-agarose column, indicating that each of them has a polynucleotide attachment site¹². By comparing the intensity of coloration of each band with a known BSA standard, we estimate that this interferon has a specific activity of at least 8×10^9 U (2.4×10^9 NIH reference units).

Our results differ in several important respects from those obtained by Knight who reported the purification of mouse interferon from L cells induced with MM virus⁸. His purified interferon preparation contained 10–11 different bands, whereas ours contained only two bands when stained with

Our results suggest, in agreement with Knight's findings⁸, that the 35,000 MW component is not a dimer of the 22,000 MW component because the electrophoretic profile did not change after boiling in denaturing conditions. Possibly the two components are different gene products, and there are some data to suggest this may be so for human leukocyte interferon¹⁸. If the two polypeptides were different gene products, the proportion in which they are produced could well depend on the cell and also on the inducer used. It is also possible that the 22,000 MW component corresponds to the polypeptide part of the 35,000 MW component from which the sugar moiety has been removed. In addition, the low biological activity found between the two stained bands may correspond to the interferon peptide with intermediate degrees of glycosylation.

We have presented evidence that interferon can inhibit cell multiplication^{11,17} and have suggested that interferon has an important role in the regulation of cell division and function¹⁹. Although this has been a subject of some controversy (see ref. 11 for references), the results presented here show that there is excellent correlation between the antiviral and cell multiplication inhibitory activities of our pure mouse interferon. Knight⁸ has reported that purified human fibroblast interferon also inhibited the multiplication of human fibroblasts. Now that mouse and human interferon have been purified it will be important to determine whether other biological effects (see refs in ref. 19) observed with crude or semipurified preparations are due to interferon.

The specific activity of purified mouse C-243 cell interferon is 2.4×10^9 NIH reference units per mg of protein, which means that for a molecular weight of 22,000 interferon is active at a concentration of 1.9×10^{-15} molar. Interferon would thus seem to be one of the most biologically active of all polypeptides.

We thank A. Cachard, M. C. Hoyez, P. Nicolas, M. T. Bandu, J. Begon-Lours and J. Buwyid for technical assistance, and Drs D. Brouty-Boyé, D. Buffe, E. Knight, E. Mogensen, R. Monier, F. Pochon, C. Rimbaud, M. N. Thang and J. P. Waller for discussions and help. Some of the interferon was from Laboratoire Roger Bellon. This study was aided by La Fondation pour la Recherche Médicale Française, the Délégation Générale à la Recherche Scientifique et Technique (contracts 76.7.1481 and 75.71670), Direction des Recherches Etudes Techniques, Direction Ministérielle pour l'Armement (contract 76/342) and CNRS (grants ATP 3174 and AI 00031858). We dedicate this article to the memory of our friend and colleague Ikuya Nagata.

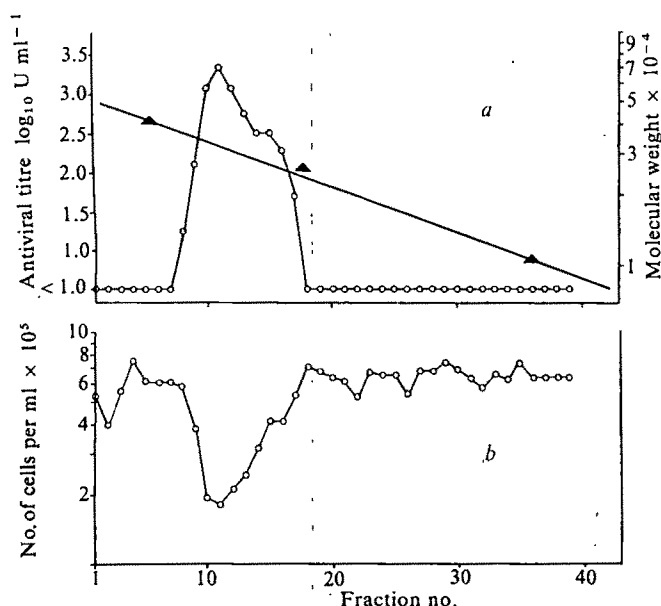


Fig. 5 Anticellular activity after elution of pure interferon from a 13% gel (with 1% β -mercaptoethanol). *a*, Antiviral activity as assayed on mouse L cells challenged with vesicular stomatitis virus. *b*, Cell multiplication inhibitory activity as assayed on mouse leukaemia L1210 cells^{11,17}. All gel eluates were incubated with 6×10^4 L1210 cells per ml in 2 ml of RPMI 1640 medium containing 10% foetal calf serum, and cell multiplication was determined after 3 d of incubation by counting viable cells in a haemocytometer and using the Trypan blue dye exclusion test. All eluates were tested at a 1:10 dilution. All points are averages of duplicate determinations. \blacktriangle , Migration of molecular weight markers: ovalbumin, chymotrypsinogen and cytochrome c.

Coomassie brilliant blue or PAS, and the interferon peaks were more pronounced and with much less dispersion of biological activity than in his study. Our 22,000 MW peak represented only 15% of total activity and stained less with PAS than did the 35,000 MW peak, in contrast to Knight's results in which the low molecular weight peak was the major component and also stained more intensely with PAS. The possibility that we have selected interferon molecules at both extremes of the molecular weight range during purification, and lost the intermediate ones, can be ruled out, because recovery from chromatography on both antibody and poly (U) was close to 100%, and in addition we showed that the interferon activity of the crude starting material had an electrophoretic profile identical to that of the purified product. These differences between Knight's results⁸ and those reported here may stem from either a greater degree of purity of our final product or differences in the characteristics of the interferon produced in the two virus-cell systems.

Received 24 October; accepted 19 December 1977.

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Structure of pyruvate kinase and similarities with other enzymes: possible implications for protein taxonomy and evolution

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The structure determination of pyruvate kinase shows that each subunit of the tetrameric molecule consists of three domains. The largest of these domains has a remarkable similarity to the structure of triosephosphate isomerase. Another domain shows similarities to many other nucleotide binding proteins. We discuss these similarities and their implications for current arguments on protein taxonomy and evolution.

We present here results from our X-ray determination of the three-dimensional structure of cat muscle pyruvate kinase (PK) at 2.6 Å resolution which may bear on questions of protein taxonomy and evolution. The X-ray data were collected photographically using an Arndt-Wonacott rotation camera¹, and phase angles were estimated by the method of isomorphous replacement with anomalous scattering measurements using three derivatives. Full experimental details will be published elsewhere.

The high resolution structures are known for about 50 proteins and certain patterns of structural similarity have appeared in a large proportion of these. Their close correlation with functional similarities has led some workers to propose evolutionary schemes for these proteins^{2,3}, although others have suggested that there are only a limited number of stable structures from which proteins are constructed^{4,5}. In the globins and some serine proteases close correspondence in tertiary and primary structure, as well as function, strongly suggests divergence from an ancestral gene^{2,3}. Amongst the dehydrogenases the sequences differ, but the dinucleotide binding domain forms a common structural feature^{6,7} and divergence has been suggested here too. The observation that the 'mononucleotide binding fold' (mnbf) defined as one half of the dehydrogenase NAD binding domain, namely three parallel strands of β sheet and two interconnecting α helices (see Fig. 2e), is common to many other nucleotide binding proteins led Eventoff and Rossmann to propose that this may be an important evolutionarily conserved unit in the kinases⁸. (We shall use the term mnbf to designate this structure without implying any binding properties).

Our studies on PK have shown that the part of the structure involved in the binding of substrates and cofactors bears a striking similarity to the structure of triosephosphate isomerase (TIM)^{9,10}, an enzyme which does not require nucleotides, and is quite unlike the known structures of the other kinases¹¹⁻¹⁴. Another part of the molecule resembles the mnbf and nucleotides bind here but in a novel fashion.

Structure of pyruvate kinase

Details of the structure of one subunit of the PK molecule are shown in Fig. 1. Each subunit comprises some 500 amino acid residues and the molecular weight of the complete molecule, consisting of four identical subunits related by twofold axes as shown, is about 240,000. The polypeptide chain is folded into three distinct domains which are shown schematically in Fig. 1. Domain A is the largest, composed of about 220 residues, and contains a cylindrical β sheet of eight parallel strands. Adjacent strands are connected by α helices which form an outer cylinder coaxial with the first. This is shown in stereo in Fig. 2a and differs from the interpretation of the 3.1-Å map in that an extra strand of β sheet has been located¹⁵. This folding pattern has been observed previously in TIM⁹. To compare these two structures a computer search procedure similar to that of Rossmann and Argos¹⁶ was carried out (Table 1 columns 5-10). A detailed comparison of the arrangement of β sheet and α helix in the two structures is shown in Fig. 2 a-d. To represent this on a two dimensional surface the α -carbon coordinates have been expressed in cylindrical polar form thereby effectively 'unwrapping' the barrel (Fig. 2 c, d). Between the third strand of β sheet and third helix of domain A the chain folds up into domain B, where about 100 residues have been located so far. As can be seen in Fig. 2, domain B occurs in the same position as the largest loop in TIM. Domain B does not seem to consist of α/β structure¹⁷, the main secondary structural feature being anti-parallel β sheet. In TIM the chain terminates at the end of the final helix of the barrel whereas in PK the chain continues for about another 120 residues forming domain C. The first section of domain C comprises two long anti-parallel α helices. The rest of the chain is folded up into a five-stranded β sheet flanked by α helices. The first three strands of sheet and two inter-connecting α helices form the well known mnbf. The third helix leads to the fourth strand of sheet and the last strand lies between strands 1 and 4 and anti-parallel to them. The remarkable similarity of part of domain C to the first mnbf of lactate dehydrogenase (LDH) is illustrated in Fig. 2e.

Comparisons of structure and function

The comparisons of these folding patterns were quantified using the computer search procedure referred to above and the results are given in Table 1 columns 5-10. This shows that the similarity in fold between TIM and domain A of PK is more significant than that between the dehydrogenases. A part of domain C agrees as well with the mnbf of LDH as does phosphoglycerate kinase (PGK), but, the mnbf's of the dehydrogenases LDH and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) agree rather better amongst themselves.

Substrate and cofactor difference maps have been published at 6-Å resolution previously for PK¹⁸. Phosphoenolpyruvate (PEP) lies at the carboxyl end of the barrel close to its axis and interacts with several large side chains. The

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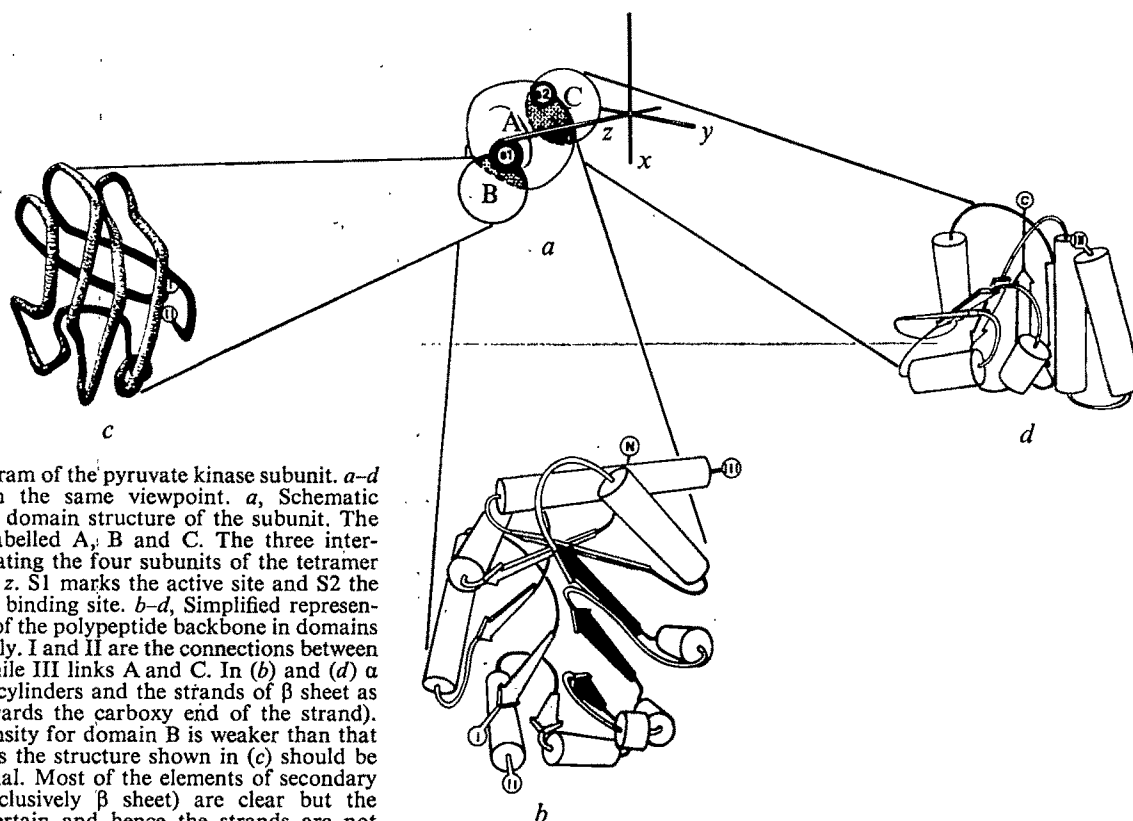


Fig. 1 Exploded diagram of the pyruvate kinase subunit. *a-d* Are all drawn from the same viewpoint. *a*, Schematic representation of the domain structure of the subunit. The three domains are labelled A, B and C. The three intersecting dyad axes relating the four subunits of the tetramer are labelled *x*, *y* and *z*. S1 marks the active site and S2 the secondary nucleotide binding site. *b-d*, Simplified representations of the course of the polypeptide backbone in domains A, B and C respectively. I and II are the connections between domains A and B while III links A and C. In (*b*) and (*d*) α helices are shown as cylinders and the strands of β sheet as arrows (pointing towards the carboxy end of the strand). Since the electron density for domain B is weaker than that for the other domains the structure shown in (*c*) should be regarded as provisional. Most of the elements of secondary structure (almost exclusively β sheet) are clear but the connections are uncertain and hence the strands are not shown as arrows.

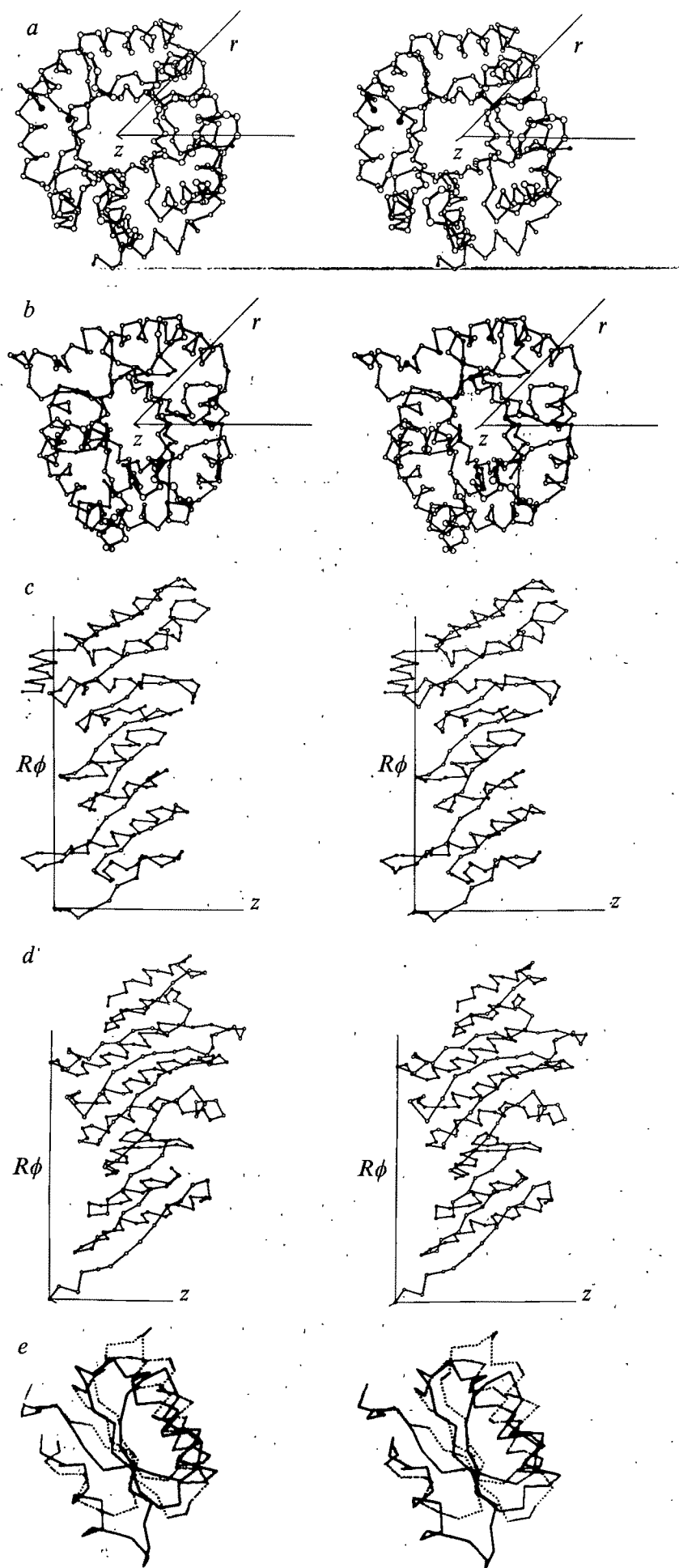
Table 1 Results of structural comparisons between domains A and C of PK and other enzymes

(1) Structure 1	(2) Structure 2	(3) Figure of topological relatedness	(4) No. of equivalent residues between 1 and 2	(5) E_1	(6) E_2	(7) % of equivalent residues in structure 1	(8) % of equivalent residues in structure 2	(9) r.m.s. deviation for these equivalents (Å)	
PK domain A	TIM	2,580,480	5,040	160	2.7	6.0	160/216 (74%)	160/247 (65%)	3.0
LDH dinucleotide binding fold	GAPDH	11,520	442	83	2.7	6.0	83/164 (51%)	83/162 (51%)	3.1
PK domain C mnbf	LDH	12	3	54	2.4	6.0	54/ 67 (81%)	54/ 76 (71%)	2.7
PGK mnbf	LDH	12	3	58	2.4	6.0	58/128 (45%)	58/ 76 (76%)	2.9
GAPDH mnbf	LDH	12	3	59	2.4	6.0	59/ 90 (66%)	59/ 76 (78%)	2.1

Columns (3) and (4): these measures are limited by being based on the extant structures and do not consider the significance that, for instance, 2 structures are eight-stranded α/β barrels, whatever the connections. However, since this measure has been used before in assessing the significance of structural similarities the numbers allow our results to be related to previous discussions, on the basis of simple, explicit assumptions. Col. (3), Schulz & Schirmer²² calculated the number of distinct topologies which could be obtained by re-connecting the helices and β sheet strands of a given α/β structure. In their treatment the 'topology' is fully described by strand sequence in the sheet and the above below pattern of connections. For n strands of sheet there are $n!$ arrangements and 2^{n-1} ways of arranging the interconnecting helices above or below the sheet. Of these topologies half are superimposable on the other half by twofold rotation. Thus there are $n! \times 2^{n-2}$ distinct topologies. Col. (4), Sternberg and Thornton⁶, and Richardson⁴ have pointed out that sheet and helix in α/β structure are nearly always connected in such a way as to form a right-handed spiral. This reduces the number to approximately $n!/2$. For an eightfold barrel, assuming that the helices, for steric reasons, cannot be on the inside, we can choose the first strand anywhere. There are then seven choices for the second and so on. There are thus $(n-1)!$ distinct topologies. Since the barrel is a closed structure the restriction of Sternberg and Thornton does not affect this total. Cols (5)–(10), structure 2 is rotated through all possible angles and compared to structure 1 for each position (Rossmann and Argos¹⁰). The likelihood that residue i in structure 1 is equivalent to residue j in structure 2 is expressed as the probability $P_{ij} = \exp(d_{ij}^2/E_1^2) \exp(S_{ij}^2/E_2^2)$. d_{ij} = the distance between $c_{\alpha(i)}$ and $c_{\alpha(j)}$ in the current orientations of the molecules $S_{ij}^2 = (d_{ij} - d_{i+1,j+1})^2 + (d_{ij} - d_{i-1,j-1})^2$ and gives a measure of how similar the shapes of the chains are on either side of $c_{\alpha(i)}$ and $c_{\alpha(j)}$. By varying the values of E_1 and E_2 we can adjust the relative weights attached to each of these two measures in assessing the overall similarity of two stretches of polypeptide chain. P_{ij} is calculated for all values of i and j (ref. 23). We choose that set of (i,j) for which the sum $\sum P_{ij}$ ($P > 0.05$) is maximum and i and j both increase from one pair to the next along the chains. Equivalent residues are then those residues which have $P_{ij} > 0.05$, the physical significance of this cut off being determined by the particular values that are used for E_1 and E_2 . Orientation and translation parameters are found for which the number of equivalences is largest²⁴. These numbers are in Col. (5) and expressed as percentages of each structure in Col. (8) and (9). The rotation matrix which brings TIM (protein data bank coordinates) into the same orientation as PK is:

$$\begin{array}{ccc} -0.7376 & 0.5956 & -0.3182 \\ -0.5214 & -0.2029 & 0.8288 \\ 0.4291 & 0.7773 & 0.4602 \end{array}$$

Fig. 2 Stereo diagrams showing the results of structural comparisons of pyruvate kinase with other enzymes. The α -carbon backbone is represented. The pyruvate kinase coordinates are as measured from a 1 cm/ \AA skeletal model. *a*, Domain A of PK. *b*, TIM, rotated so as to give the overall 'best fit' as defined in Table 1 when superimposed on domain A of PK. Note that the cross section of the barrel is more elliptical than in PK. *c*, Domain A of PK unwrapped. The cartesian coordinates of the α carbons have been transformed into cylindrical polar coordinates r , z and Φ where the cylindrical polar axes are as shown in (*a*). r is the distance along the azimuthal vector, Φ is the azimuthal angle and z is the distance along the cylinder axis. R is the approximate radius of the β sheet cylinder. $R = 8.5 \text{ \AA}$. *d*, TIM unwrapped as in (*c*). $R = 8.0 \text{ \AA}$. This shows clearly any small differences in the orientation of the structure elements. *e*, The mnbf LDH (dotted line) compared to part of domain C of PK (full line).



density for Mn-ATP lies radially perpendicular to the barrel axis so that the end of the density interpreted as the terminal phosphate overlaps the PEP binding site and the other end is between strands 3 and 4 of the barrel β sheet. This is unlike PGK where the 3-phosphoglycerate (N. Walker, personal communication) and nucleotide^{11,12} lie along the end of the β sheet. However, the PEP density is in a similar position to that of the substrate dihydroxyacetone phosphate (DHAP) in TIM⁹ (see Fig. 4). In suitable conditions ADP can be bound at a site between domains A and C in such a position that it interacts with the amino ends of β strands of the barrel and the carboxy end of the second helix of the mnbf in domain C. Surprisingly it is not at the carboxy end of the β sheet of this structure (Fig. 1).

Since kinases are grouped together on the basis of phosphoryl transfer from ATP the similarity between domain A of PK and TIM is at first sight unexpected. However, there are in fact similarities in the reactions of the two enzymes if we consider enolisation of the 2-keto-triose substrates. In PK, PEP is produced by deprotonating and phosphorylating pyruvate, while in TIM the formation of glyceraldehyde-3-phosphate is accomplished through deprotonation of dihydroxyacetone phosphate. It has been proposed that in each case a base on the enzyme participates in the deprotonation reactions. The existence of a *cis*-enediol intermediate has been demonstrated for TIM^{19,20}. Rose has shown that in the reaction catalysed by PK, enolisation of pyruvate may be decoupled from phosphorylation²¹. Figure 3 illustrates the similarity in this part of the proposed mechanisms.

We have already noted that the substrate binding sites are similarly located on the axis of the barrel in both PK and TIM. The next question to ask is are the active sites of the two proteins similar? Since the primary structure of PK is unknown we cannot identify the side chains with any certainty. We present the side chains which appear in the electron density map in the region where substrates bind, together with the corresponding region of the TIM model (Fig. 4). From this diagram it seems that there are side chains in the active sites of both PK and TIM that are associated with corresponding β -sheet elements. As far as can be seen the sizes and orientations are similar. Thus, as well as comparing PK with the other kinases, it may be useful to include consideration of the fine differences between PK and TIM in studies of the mechanisms of these two enzymes.

Fig. 3 The proton transfer reactions catalysed by pyruvate kinase and triose phosphate isomerase. In each case B is a base on the enzyme. Enol-pyruvate can be considered to be an enzyme-bound intermediate in the PK reaction²⁶ and a *cis*-enediol intermediate has been implicated in the TIM reaction²⁰.

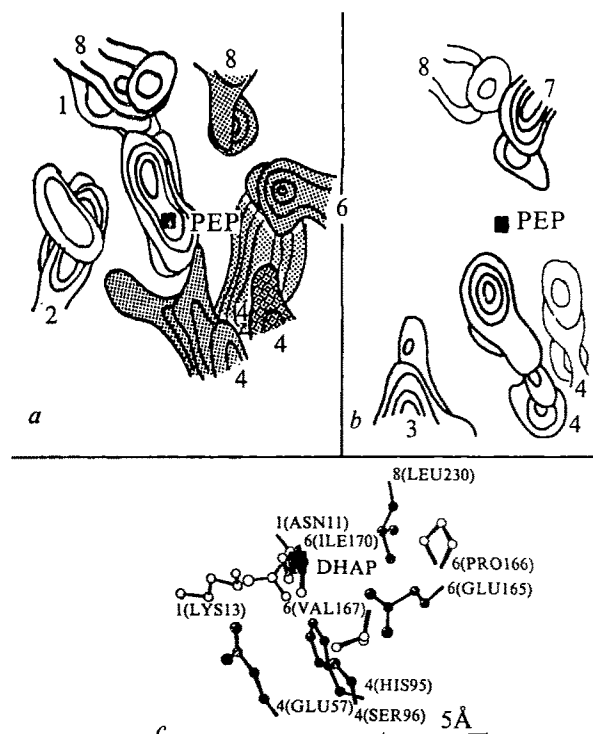
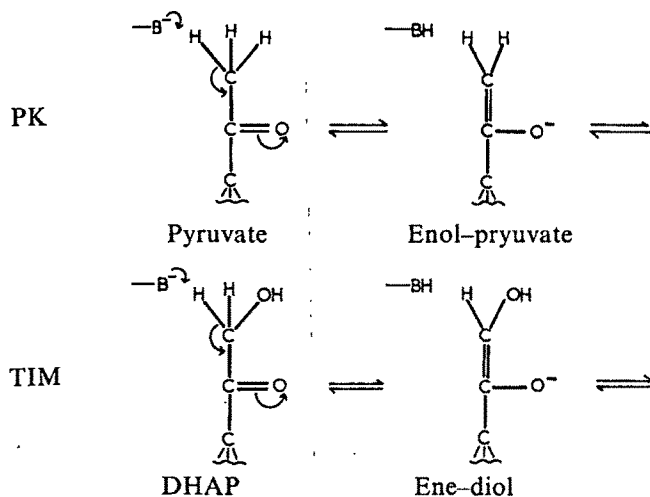


Fig. 4 *a, b*, Pyruvate kinase active site. The electron density corresponding to side chains close to the binding site of PEP is shown, this is the area marked S1 in Fig. 1. The direction of view is along the axis marked y in Fig. 1 with the z axis horizontal. Each side chain is numbered to indicate which element of the β -sheet barrel it is attached to. The n th element of the barrel is defined as containing all residues between the beginning of the n th strand and the end of the n th helix. In the electron density map, the unit cell of the crystal is sectioned perpendicular to the y -axis. The thickness of each section is 1.25 Å. The PEP site is centred on section 38 and is marked ■. (*a*) Shows a composite of sections 32 to 38 and *b*, of sections 38 to 42. The side chains only are shown, the main chain having been left out for clarity. *c*, Triosephosphate isomerase active site. The orientation corresponds to that of (*a*) and (*b*) and the atomic coordinates for the side chains of the residues close to the DHAP position are shown. C₂ of DHAP is marked ■. The labels show the element of barrel to which each residue is attached with the residue type and number given in parenthesis. The scale of this diagram is the same as (*a*) and (*b*). The residues shaded in (*a*) seem to occupy analogous positions to those with filled atoms in (*c*) and it will be seen that the secondary structural elements that they are attached to correspond. Phillips *et al.*²⁶ have shown that the proposal, based on the X-ray diffraction results, that Glu 165 is the base implicated in the mechanism (Fig. 3) is consistent with other biochemical evidence. In comparing the two diagrams note that the barrels are not exactly the same shape in TIM and PK (Fig. 2*a, b*) and consequently groups attached to different strands are displaced relative to each other. Note also that three of the residues of the fourth sheet-helix element seem to be in similar relative positions in the two structures. These three residues in TIM²⁶, His 95, Ser 96, Glu 97, are all members of the group of residues implicated in the active site.

Pyruvate kinase and evolution

Our results suggest that domain A of PK and TIM may have evolved from a common ancestor but contradict the idea⁵ that the appearance of the mnbf in an enzyme implies the binding of a nucleotide in the same fashion as in the dehydrogenases and its evolutionary conservation for this function alone. Thus if one considers that any three consecutive strands of the eightfold barrel comprise a mnbf⁹ then the two nucleotide binding sites in PK are both associated with a mnbf. However, the manner of binding is quite different to that found in the dehydrogenases. At the active site the nucleotide is bound at the carboxy end of the β sheet as in the dehydrogenases but is orientated along a radius instead of along the edge of the sheet. At the secondary site it is not even near to the β sheet. It could be

argued^{4,5} that a structure like the mnbf is likely to form in PK if only because it is so highly probable in such a large α/β structure. We feel that although this may be true it tends to divert attention from interesting considerations of structure and function and their possible relation to evolution. It may be that proposals for simple evolutionary relationships between structures should at present be confined to functional domains as in the dehydrogenases or domain A of PK and not applied to stable substructures such as the mnbf which could conceivably be dissected out from them. If the similarities between domain A and TIM do imply a divergent evolutionary relationship rather than convergent evolution then it would seem that either PK is a composite of parts which had separate evolutionary histories before their joining together to make the modern enzyme or TIM is derived from a fragment of an ancestral protein. We would still have to account for the derivations of domains B and C in PK. It is also apparent that the kinases do not form such a closely related group of structures as the dehydrogenases. All known kinase structures consist of α/β units¹¹⁻¹⁴. PGK^{11,12} possesses the 'dinucleotide binding fold' of LDH and apparently binds the nucleotide in a similar manner. PK, hexokinase¹⁴ and adenyl kinase¹³ all differ.

The basic difference between the PK/TIM homology and those observed previously is that whereas for, say, the dehydrogenases the function of the postulated conserved gene is characteristic of a group of enzymes accepted as belonging to a single class, in the PK/TIM case, the function conserved would cut across the two categories 'kinase' and 'isomerase'. As more protein structures are revealed the present categories may therefore be found

inadequate and other groupings may emerge which reflect both similarities of domain structure and the different aspects of the function. The problem posed by the structural diversity of the enzymes now classed as kinases may then disappear.

We thank Dr John Williams for helpful discussions, Dr I. A. Wilson and Professor D. C. Phillips for use of unpublished results, the Protein Data Bank for atomic coordinates of proteins and the SRC for support.

Received 26 September; accepted 8 December 1977.

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letters to nature

Dual character of the rapid burster and a classification of X-ray bursts

DIFFERENT kinds of X-ray bursts, as observed from the rapid burster (MXB1730-335) (refs 1, 2) and from other sources³⁻⁵, can be classified into two types which, as we will show, may have different origins and production mechanisms. Type I bursts occur at intervals of hours, days or longer. Their spectra almost always soften during burst decay, and their average spectrum during the first few seconds of a burst is generally harder than the spectrum of the associated persistent X-ray emission (if present). Type II bursts occur at intervals of several seconds to minutes, and their spectra do not soften during burst decay. The rapid burster¹⁻⁶ can produce up to several thousand type II bursts per day. Type II bursts may perhaps also be produced by other X-ray sources such as Cyg X-1 (ref. 7). Type I bursts are produced by all other burst sources reported so far³⁻⁵. Here we will show that the rapid burster also produces type I bursts.

Between 1977 September 27.27 and October 2.76 UT we observed the rapid burster (MXB1730-335) (ref. 1) with the y -axis detectors of the SAS-3 observatory⁸. The burst rate varied from $\sim 50 \text{ h}^{-1}$ to $\sim 250 \text{ h}^{-1}$, the burst durations from $\sim 3 \text{ s}$ to $\sim 10 \text{ s}$ (ref. 9).

In addition to the rapidly repetitive bursts (up to several thousand per day), henceforth called 'rapid' bursts, we detected 19 bursts which have a different appearance: (1) they

last longer ($\sim 30 \text{ s}$) than the rapid bursts; (2) they occur out of sequence of the rapid burst pattern, grossly violating the $E-\Delta t$ relations for rapid bursts^{1,4,6}; (3) their spectra soften during burst decay unlike the spectra of the rapid bursts; and (4) their time-averaged spectra during the first $\sim 10 \text{ s}$ are often harder than those of the rapid bursts.

Figure 1 shows, in addition to many rapid bursts, three of the 19 'special' bursts which were recorded in the horizontal tube system⁸ (1.3-12 keV). In several cases we detected a rapid burst coincident with a special burst (Fig. 1b and c), as would be expected (chance coincidence) if the two are unrelated. This may indicate separate origins and possibly different mechanisms for the two types of bursts.

Based on the similarity of the relative counting rates in the independent y -axis detectors for the special and rapid bursts, the 19 special bursts must have come from within $\sim 10 \text{ arc min}$ of MXB1730-335. We believe that they actually came from MXB1730-335. They did not come from MXB1728-34 (at $\sim 0.5^\circ$ distance)¹⁰ as we detected 11 bursts from this source with markedly different ratios of the counting rates in the y -axis detectors than the special bursts.

Figure 2 shows the data from five energy channels of the bursts shown in Fig. 1a. The softening of the spectrum during the decay of the special burst is clearly visible, but it is absent in the five rapid bursts. Also, unlike the rapid bursts, the special burst is very distinct in the 8-19 keV channel.

Table 1 lists the times when the 19 bursts were detected. The burst intervals between the detected bursts are plotted in Fig. 3.

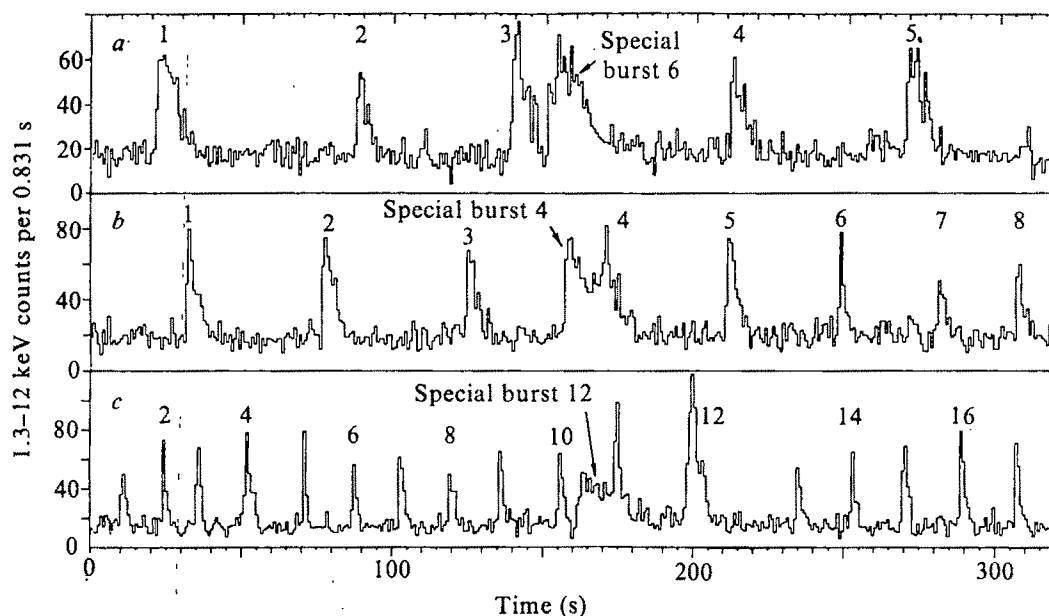


Fig. 1 Three sections of SAS-3 data from the horizontal tube detectors (sum of three channels, 1.3–12 keV). Each section contains a 'special' burst: *a*, burst 6; *b*, burst 4; *c*, burst 12 (see Table 1). The counting rates would have been somewhat higher if the rapid burster had been exactly in the centre of the field of view. Correction factors for the burst data are 1.11 (*a*), 1.05 (*b*) and 1.18 (*c*). The special bursts (type I) arrive out of sequence of the rapidly repetitive bursts (type II), which are independently numbered. They have approximately the same height as the 'rapid' bursts, but they last considerably longer (~30–40 s). In (*b*) and (*c*) a rapid burst occurs simultaneously with a special burst. Note in (*c*) that the special burst (12) is followed by a large rapid burst (no. 12) ~45 s later. Such a coincidence was observed four times (see text).

The minimum detected interval was 2.9h, the maximum was 20.4h. Some bursts were almost certainly missed due to Earth occultation.

There are no obvious regularities in the burst intervals (Fig. 3), but it is possible that a few semi-regular series were present with intervals near 3–4h (see dashed lines in Fig. 3). The regularity was clearly broken at times. We should point out that the burst intervals can, during certain periods, become synchronised (in phase) with the 30 min occulted portion of each 95 min orbit. For example, the mean burst intervals between bursts 8 and 13 (day 416–417 in Fig. 3) may have been ~3.1h (r.m.s. jitter 15%), which is almost exactly twice the orbital period (1.57h). Therefore, the probability of missing four bursts in a row (near day 418; Fig. 3) is not necessarily low. Nevertheless, to fit all 19 bursts in one semi-regular series with intervals of ~3–4h, spanning the entire 5.5d observing period, would require at least 18 bursts to have been blocked by the Earth; this probability is negligibly small.

Between September 30.0 and 30.8 (UT) 1977, the rapid burst rate was very high, ~160–220 bursts h^{-1} , compared to ~95 bursts h^{-1} averaged over the rest of the observation. During this period, the bursts were nearly equal in size, except that in every orbit, 1–4 larger rapid bursts were emitted (average ~2.6 h^{-1}) with 3–5 times more energy than the other bursts. Such large bursts were occasionally detected during the rest of the observation, but only in ~10% of the orbits. Of 45 such large rapid bursts detected in the entire 5.5 day observation, 25 were detected in the September 30.0 to 30.8 period. During this ~19 h period (~12.7 h of unocculted data on the rapid burster) we detected four special bursts (10–13 in Table 1), each of which was followed within ~80 s by such a large rapid burst (Fig. 1c). Given the observed rate of special bursts (~0.32 h^{-1}) and of large rapid bursts (~2.6 h^{-1}) during this interval, the expected number of such close coincidences is ~0.02 h^{-1} , or ~0.25 during the entire interval. The probability of observing four such close coincidences is thus ~ 10^{-4} . We therefore conclude that there is some causal connection between the special bursts and the rapid burst, and thus that the special bursts came from MXB1730–335.

The rapid bursts from the rapid burster are of type II, and are different from type I bursts in that they occur at a much higher

rate and do not show spectral softening during burst decay. The 19 special bursts are type I bursts. They resemble the bursts observed from many other sources^{3–5} in that: the time intervals between bursts (hours) are comparable; the burst recurrence patterns are similar (refs 5, 11, 12 and SAS-3 unpublished results); and the softening of the spectra during burst decay observed in the special bursts is characteristic of X-ray bursts from almost all sources^{3–5}.

The detection of type I bursts from the rapid burster may indicate that the rapid burster, in spite of its unique behaviour, is physically similar to other burst sources.

We have examined our March–April 1976 data from the rapid burster for the presence of special bursts. We found none in the March data but we found six bursts in the April data that are very similar to special bursts. They were in a semi-regular sequence with average burst intervals of ~3.8h (r.m.s. jitter 10%), and the spectral softening during burst decay was very clear in at least two cases. The horizontal tube xenon detectors⁸ were turned off at the time; we therefore cannot tell whether the spectra of the six bursts were harder than that of the rapid bursts.

The rapid burst pattern was very similar in April 1976 and September–October 1977 (regular burst intervals) but very different in March 1976 (intervals differed by a factor of ~100) (refs 1–4). This may well be why special bursts were detected

Table 1 Times that special (type I) bursts were observed from MXB1730–335 between 1977 September 27.267 and October 2.760 (UT).

13.767 (Begin observation)			
1	13.9343	11	16.7352
2	14.2800	12	16.9575
3	14.7179	13	17.2161
4	14.8657	14	18.0655
5	14.9983	15	18.3693
6	15.1866	16	18.5970
7	15.7575	17	18.7156
8	16.0347	18	18.8616
9	16.3023	19	19.1000
10	16.6015		19.2596 (End observation)
Julian Day—2443400 days			

A type I burst occurring at 18.9854 UT was discovered in the data after this letter was submitted. This is not included in this table or in Fig. 3.

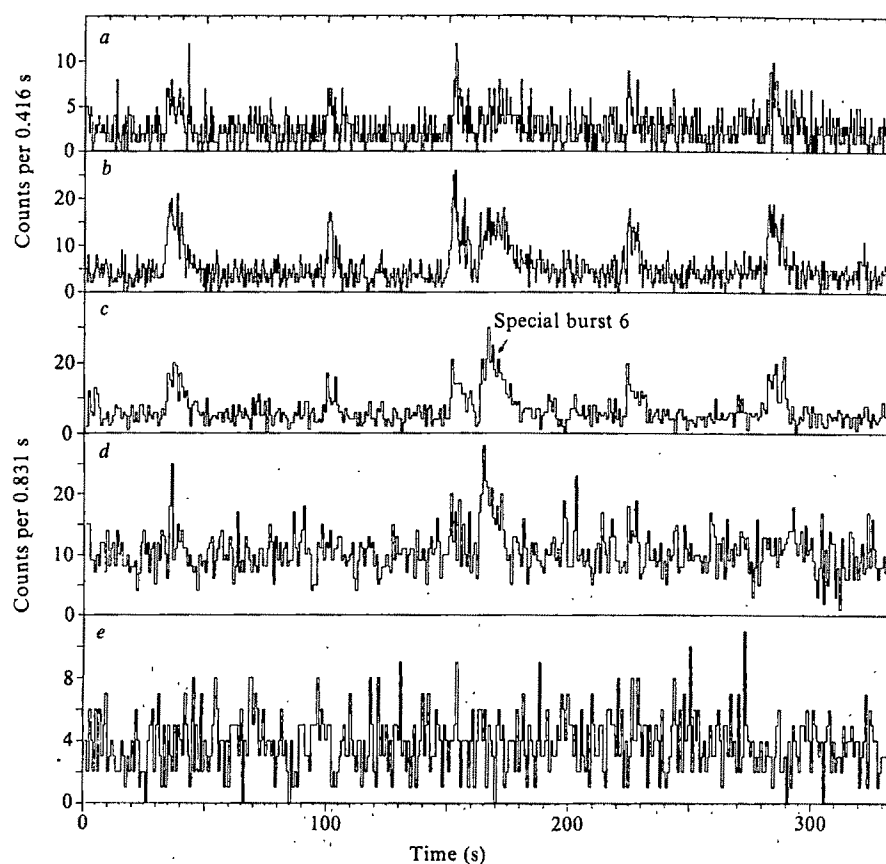
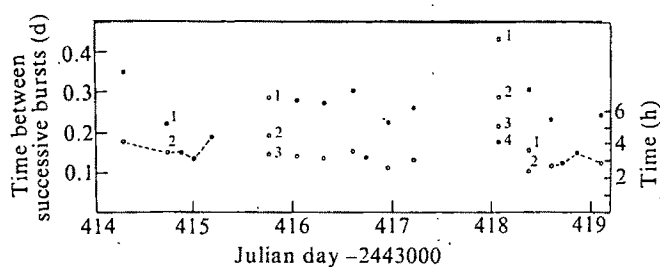


Fig. 2 Spectral information (five energy channels) for the data shown in Fig. 1a: a, 1.3–3 keV; b, 3–6 keV; c, 6–12 keV; d, 8–19 keV; e, 19–27 keV. Notice that the spectrum during burst decay of the special (type I) burst softens. This softening is very common in bursts from other sources, but it is absent in the rapid (type II) bursts. The special burst is clearly visible in the range 8–19 keV, and thus its mean spectrum (first ~ 10 s) is harder than that of the rapid bursts.

in April 1976 and September–October 1977 but not in March 1976. We did, however, detect 23 unusual bursts in March 1976 of which 17 were previously reported as ‘anomalous’ bursts by Ulmer *et al.*². They occurred at irregular intervals and all were preceded by a very energetic rapid burst. This established that the anomalous bursts came from the rapid burster and that there is a causal relation between the rapid and the anomalous bursts². The anomalous bursts have some features in common with the special bursts, and there are some differences. Both have durations of ~ 30 s, and both occur out of sequence of the rapid burst pattern, grossly violating the $E-\Delta t$ relations for rapid bursts^{1,4,6}. The ratio of the time-averaged flux of rapid bursts and special bursts was ~ 120 . This is similar to the ratio of ~ 140 for rapid bursts and anomalous bursts. The main differences between anomalous and special bursts are that anomalous bursts have lower peak luminosity compared to the most energetic rapid bursts, longer rise times, a softer mean spectrum,

Fig. 3 Intervals between detected bursts are shown as black dots. \circ , Indicate the observed intervals divided by two, three, four and so on (bursts can be missed as a result of Earth blocking). The numbers next to the open circles indicate the number of possibly missing bursts. Semi-regular burst series may have occurred near day 415 (dashed line), between day 416 and 417, and near day 419 (dashed line) (see text).



and they probably lack the spectral softening during burst decay (although, because anomalous bursts are relatively weak, this latter point is not certain). We do not know whether anomalous and special bursts are related in that they are caused by the same phenomenon but we suspect that they are.

White *et al.*⁶ have discussed the previously well known breakdown of the linearity in the $E-\Delta t$ relation of rapid bursts (first reported by Lewin in December 1976) (ref. 4). They show four days of data of the rapid burster as observed in April 1977 with Ariel V. There are no special bursts in their data. Anomalous bursts might be present in the Ariel V data. But, the sensitivity of the Ariel V observations is probably too low to recognise them as the peak luminosity of anomalous bursts is $\sim 1/3$ that of rapid bursts² (but see note added in proof).

The rapid burster seems to have a dual character. On one hand, it can produce type I bursts similar to those observed from other burst sources. On the other hand, quite independently, it can produce type II bursts in rapid succession (up to $\sim 4,000$ per day). Bursts of type I and type II can occur simultaneously (Fig. 1b and c) which may indicate separate origins and possibly different mechanisms for their production. The two types of bursts can influence one another, however.

In order to find out whether there are times when the rapid burster produces only type I bursts, we examined several weeks of data from the nearby source MXB1728–34, during times that no rapidly repetitive bursts were observed from MXB1730–335 (refs 13–15 and SAS-3 unpublished results). We did not find any. This, of course, does not prove that it never happens, and we plan to observe the rapid burster frequently after the rapidly repetitive type II bursts have ceased. This is expected some time in November 1977, if the duration of rapid burster activity is the same as was observed in the springs of 1976 and 1977 (refs 1, 6, 15–17).

The ratio of the time-averaged luminosity of rapid bursts

(type II) to that of both 'special' bursts and 'anomalous' bursts (type I) was ~ 130 . This is comparable to the ratio of time-averaged persistent X-ray emission and time-averaged type I burst emission for many burst sources³⁻⁵. This opens up the interesting possibility (suggested by J. Doty, personal communication) that in MXB1730-335 the persistent X-ray emission as observed from many burst sources manifests itself in the form of rapidly repetitive type II bursts. In support of this, we point out that the mean burst spectra of rapid bursts are similar to the spectra of the persistent emission from most sources that produce bursts³⁻⁵. Also, the mean spectra (first ~ 10 s) of special (type I) bursts are harder than those of the rapid (type II) bursts just as the mean spectra of type I bursts from most sources are harder than that of the persistent emission³⁻⁵.

If this idea is correct, it may remove one of the main problems with thermonuclear flash models of X-ray bursts (ref. 5 and references therein), which predict the ratio of time-averaged steady luminosity to time-averaged burst luminosity to be $\lesssim 10^2$. No persistent X-ray emission has been detected from the rapid burster^{1,6}, and the ratio of steady luminosity to time-averaged type II burst luminosity has been measured to be as low as < 0.2 (ref. 6). But, if the rapidly repetitive type II bursts take the place of the steady emission and type I bursts have a thermonuclear origin, then the equivalent ratio would be ~ 130 for MXB1730-335.

Type II bursts as observed from the rapid burster are probably the result of instabilities in the accretion flow. The more common type I bursts may be the result of thermonuclear flashes or they too could result from some kind of an instability in the accretion flow (ref. 5 and references therein).

The brief flares as observed from the binary sources Cyg X-1 (ref. 7), GX304-1 (ref. 18) and LMCX-4 (ref. 19) meet the phenomenological definition of type II bursts. We do not discount the possibility that those flares are produced by the same mechanism that produces type II bursts in the rapid burster.

We thank Lynn Cominsky, John Doty and Claude Canizares for helpful discussions.

Note added in proof: We are informed by K. Mason (personal communication) that Ariel V would have detected anomalous bursts of the strength reported by Ulmer *et al.*² if they had occurred. Therefore, the rapid burster was producing neither special nor anomalous bursts in April 1977.

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Received 14 November; accepted 14 December 1977.

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The nature of Aquila X-1

THE unusual X-ray source Aquila X-1 (=4U1908+00; =2S1908+005) has been observed for almost a decade, and represents a singular link between the 'steady' galactic X-ray stars and the flaring and transient sources. Although this object normally has an X-ray flux of the order 1-10% of the Crab Nebula¹⁻³, approximately once per year it undergoes an intense outburst to a level comparable to the Crab²⁻⁴. A 1.3-d X-ray periodicity during the outburst has also been suggested⁵. A recent very accurate X-ray position for Aql X-1⁶ has permitted the optical identification of the source⁷ using primarily the extensive plate material described by Davidsen *et al.*². We report here photometry and spectroscopy of the counterpart and the stars in the immediately surrounding field, and use these data to arrive at some inferences regarding the nature of this unusual X-ray system.

Photoelectric photometry of nine stars in a 1 arc min field about Aql X-1 was obtained in July and August 1976, using the 2.1 m reflector of the Kitt Peak National Observatory with the Cassegrain computer-photometer. Standardisation of these *UBV* data was achieved through observations of standard stars from the compilation of Landolt⁸; the objects observed were in the range $12.4 < V < 17.1$, $13.1 < B < 18.3$. Spectroscopy of nine stars in the field, partially overlapping with the photometric sample and ranging $12.4 < V < 15.2$, was obtained in November 1975 using the Mk I multichannel spectrometer⁹ at the 1.3 m reflector of the McGraw Hill Observatory. Spectral types were derived for these stars through comparison with spectral standards¹⁰ and through H α equivalent widths¹¹.

We have also obtained photographic photometry of the optical counterpart in quiescence and outburst. The quiescent data consist of direct photographic plates obtained with the 0.9 m Crossley reflector of the Lick Observatory in August and October 1977. These plates were calibrated through iris photometry of the stars in the photoelectric sequence described above, providing a direct comparison sequence extending to $V=17$, $B=18$, and through a transfer from the M15 field described by Sandage¹², which is photoelectrically calibrated to $V=22$, $B=23$. This combined photoelectric-photographic calibration of the Aql X-1 field is considerably more accurate than a standard photographic transfer, judging from the scatter in the magnitudes derived for the standard stars, which is of the order 0.10-0.15 mag even for the faintest objects. The outburst data consist of the Palomar Observatory Sky Survey prints which remarkably show Aql X-1 near maximum light on the date (August 23-24 1951) they were exposed. The *B* and *R* magnitudes of Aql X-1 on these prints were determined by measuring on a 2-axis Mann measuring engine image diameters of the comparison stars discussed above.

We find that for the Aql X-1 counterpart in quiescence, $V=19.4 \pm 0.15$, and $(B-V) \gtrsim 1.0$; a *B* image is only barely detectable on our plate material. During outburst we find $B=18.41 \pm 0.04$, $R=17.37 \pm 0.24$. Using a colour equation applicable to the Sky Survey¹³, we infer $(B-V)=0.7 \pm 0.2$. An uncalibrated outburst plate described by Thorstensen¹⁴ indicates that the full range of variability may in fact be slightly larger, as in the *B* band the counterpart reached a brightness comparable to star no. 3 in the chart of ref. 6. For that star our photoelectric data yield $B=17.8$. Assuming a reasonably constant colour near the outburst peak, we adopt $V=17.1$, $(B-V)=0.7$ for the outburst state. The large range of inferred photometric variability, together with the simultaneity of two X-ray and optical outbursts⁷ makes the identification of the optical counterpart (star 5 in ref. 6) secure.

Our photometry and spectroscopy of the stars in the immediately surrounding field yield information on the interstellar extinction in this direction. For the stars with spectroscopy, we used our photoelectric or photographic magnitudes and colours to estimate colour excesses and distances; six of

these objects prove to be foreground K stars and thus not distant enough to provide a useful probe. For three additional stars with photoelectric *UBV* data but no spectra, the 'Q' method¹⁵ indicates *B* spectral types, again permitting extinction and distance estimates. The six stars for which we have useful data span a range in distance of 0.3–5 kpc and in A_V of 1.5–4.4 mag. We find these data to be consistent with an exponential model¹⁶ for the distribution of dust perpendicular to the galactic plane, and with the parameters given by Allen¹⁷ of $A_V/d = 1.9 \text{ mag kpc}^{-1}$ in the plane and scale height $H = 130\text{--}190$ pc. The line of sight to Aql X-1 breaks out of the dust layer at a distance of 1.9–2.7 kpc and has a limiting visual extinction A_V of 4–5 mag in this model.

Using this model for the interstellar extinction, we have transformed our observed brightness and colour of Aql X-1 during quiescence and outburst to luminosity and intrinsic colour as a function of assumed distance. The spectral observations¹⁴ suggest a quiescent spectral class near K0. Our quiescent brightness data place dwarf stars of this approximate spectral class at distances between 1 and 2 kpc. Giant stars of the observed spectral class are ruled out by the colours and our reddening against distance relation. In particular, a K0 dwarf at a distance of 1.6 kpc is in good agreement with all the data, and implies an outburst X-ray luminosity far below the Eddington limit.

The existing data, though fragmentary, have interesting implications. The absence of emission lines in the quiescent spectrum¹⁴, despite the detection of an absorption feature, emphasises the difference between Aql X-1 and the dwarf novae, which have emission spectra at all times. This is in contrast to the suggestion⁴ that Aql X-1 may be similar to the dwarf novae. Most striking, however, is the high ratio of X-ray to optical intensity. At maximum light, using our *V* magnitudes and the observed X-ray fluxes of Kaluzienski *et al.*⁴, we find for this ratio a value of about 5,000; in the quiescent state, the ratio is lower but still at least 500. These values should be compared to other identified X-ray sources; the largest value is probably that of Sco X-1, which has a ratio of about 1,000 (see ref. 18). Aql X-1 is even more outstanding than this extra factor of 5 indicates, because in Sco X-1 the visible light source has a temperature $\sim 30,000$ K and a bolometric correction of several magnitudes, probably reducing the bolometric X-ray to optical ratio to $\sim 10^2$. Our colour measured for Aql X-1, together with the indication¹⁴ of a late G or early K type spectrum when quiescent, suggests a reddening $E(B-V)$ of a few tenths of a magnitude and a small bolometric correction during outburst. Correction for extinction decreases the outburst X-ray to optical ratio, but probably not below 500.

The fact that the quiescent spectrum¹⁴ is a late-type absorption spectrum implies that most of the observed optical luminosity is generated within the star. The photospheric opacity of a K dwarf, for example, is about $0.1 \text{ cm}^2 \text{ g}^{-1}$, less than the opacity of matter to any X-rays. Therefore X-ray heating sufficient to substantially affect the atmospheric heat balance during quiescence would produce a strong chromosphere and an emission spectrum, as in Sco X-1^{18,19}; the absence of these implies that the atmosphere is heated from within the star. This is consistent with the fact that the X-ray brightness increases an order of magnitude more than the visible brightness from quiescence to flare.

An X-ray to optical luminosity ratio of 500 implies, in the conventional semidetached Roche binary model, that the companion's radius is about 0.1 of the separation²⁰; equivalently, the X-ray source is about 100 times more massive than its companion. This conclusion is independent of the distance, except through the extinction, but requires that the X-ray radiation should not be highly anisotropic. Three possible solutions to the problem posed by the small size of the companion are a companion of less than stellar mass, a supermassive X-ray source, or abandonment of the assumption that the system is semidetached. The first is ruled out by the fact that in quiescence the companion's luminosity comes

from within, and the second is possible, but will be thought speculative by most. The third explanation, which we favour, poses the problem of how mass transfer is brought about. Oke²¹ argues that the X-ray transient A0620–00 (=V616 Mon; =Nova Mon 1975) is also a K dwarf and presents a similar problem.

If our suggested model for Aql X-1 is correct, it should show (for favourable inclination) a strong photometric periodicity during outburst, perhaps resembling HZ Her, but not when quiescent. During outburst, there may also be an emission spectrum, as in Sco X-1 and A0620–00. There could be a detectable spectroscopic orbit. With our estimated binary separation, a total system mass of $2M_\odot$ and a dwarf companion, an orbital period of about 2 d is implied, possibly consistent with the 1.3-d X-ray period reported by Watson⁵.

Thus, perhaps the most important implication of the data is that Aql X-1 may be the second known case of a low mass galactic X-ray source where the semi-detached Roche model, previously widely used for these systems, is not applicable. We also have strong evidence that the primary is a dwarf and that X-ray heating is the source of the optical outbursts.

We are indebted to E. A. Harlan for photographic observations. J.I.K. is an Alfred P. Sloan Research Fellow. This work was supported in part by the U.S. National Aeronautics and Space Administration.

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γ -Rays from γ Geminorum?

THE γ -ray source CG195 + 4 (Geminga) discovered by SAS 2 and further observed by COS B has a period of 59 s which is increasing at a rate $\dot{P} = 2 \times 10^{-9}$ (refs 1,2). If the periodicity is produced by a neutron star which is spinning down at the observed rate, then the rate at which rotational energy is being lost is $L \sim 3 \times 10^{32} \text{ erg s}^{-1}$ (ref. 3). The observed γ -ray flux of $3 \times 10^{-6} \text{ photon cm}^{-2} \text{ s}^{-1}$ with energy $E > 100 \text{ MeV}$ implies that the distance, d , to the source is given by $d \approx 73 f^{1/2} \text{ pc}$ where $f (\geq 1)$ is the fraction of the total energy output which is emitted as γ -rays. Theoretical studies

of the evolution of binary systems which give rise to the binary X-ray sources have led to the suggestion that, before the neutron star starts to accrete and spin up (that is, before the X-ray source turns on) it spends a comparatively long time being spun down in the weak stellar wind of its companion⁴. This leads us to seek a candidate companion for the γ -ray source which is an early type star at a distance of $\gtrsim 70$ pc. As we report here, the only object which satisfies these constraints is γ Geminorum.

The star γ Gem has $m_v = 1.9$ mag and has been classified as A0IV (ref. 5) and A1III (ref. 6). Parallax measurements yield a distance estimate of $d \sim 30$ pc (ref. 7). We therefore require $f \sim 0.17$. We assume the star to have a bolometric luminosity $L_{\text{bol}} \sim 6 \times 10^{35}$ erg s⁻¹, a mass $M_1 \sim 3M_{\odot}$ and a radius $R_1 \sim 3.5 \times 10^{11}$ cm. If we consider a neutron star orbiting γ Gem at a distance comparable to R_1 then a substantial fraction ($\sim 1/4$) of the primary's stellar wind will be accreted towards the companion at a rate \dot{M}_A .

We assume that the neutron star is strongly magnetic and that its corotation radius ($R_{\Omega} \sim 2.3 \times 10^9$ cm) is roughly equal to the Alfvén radius of its magnetosphere, R_M . The neutron star is in its 'sleeping phase': that is, material is not accreted down to the stellar surface but is, rather, expelled from the vicinity of the Alfvén surface. The energy to do this comes from the rotational energy of the neutron star, and if the material is thrown off at about the escape velocity from the region of the Alfvén radius, $V_M \simeq (2GM/R_M)^{1/2}$, we may write $L \sim GM\dot{M}_A/R_M$. This implies that $\dot{M}_A \simeq 5 \times 10^{15}$ g s⁻¹, so that $\dot{M}_W \sim 2 \times 10^{16}$ g s⁻¹. Considering that mass loss will be enhanced by the presence of a close companion this is a reasonable value for the mass loss rate of a star like γ Gem (ref. 8). Making use of the definition of the Alfvén radius, $B^2(R_M)/8\pi \sim 1/2 \rho_M V_M^2$ where $\rho_M = \dot{M}_A/(4\pi R_M^2 V_M)$ is the density of the gas at R_M , we obtain the surface field (assumed dipolar) of the neutron star to be $B \approx 7 \times 10^{12}$ gauss. We have taken the neutron star to have mass $1 M_{\odot}$ and radius 10^6 cm.

Despite the assumed equality of R_M and R_{Ω} material does not accrete down to the stellar surface because at the low density and the high temperature to which the matter is heated ($T \sim 1/2 m_p v_M^2/k \sim 7 \times 10^8$ K) it cannot cool sufficiently by bremsstrahlung to accrete^{9,10}. The energy loss rate due to bremsstrahlung radiation is about 10^{30} erg s⁻¹ which is much less than L , the rate of energy generation at the magnetosphere. This energy must take the form of bulk motion of the gas and hence the gas is expelled.

To produce the γ -rays we suggest that a fraction of the energy released in the interaction between the gas and magnetic field at R_M is in the form of relativistic electrons with $\gamma \sim 2 \times 10^3$. If about a half of these penetrate the magnetosphere, where they radiate by synchrotron in the extreme ultraviolet (~ 400 Å), the dominant loss process for the remainder is inverse Compton scattering on these ultraviolet photons, resulting in the production of the observed 100 MeV γ -rays. The electron energy-loss timescale for this process, t_{IC} , is less than half the pulse period if the electrons are accelerated only over a fraction $\gtrsim 1/4$ of the magnetosphere. This seems likely, given a non-axisymmetric magnetosphere and, together with asymmetries in the initial velocity distribution of the electrons producing the γ -rays, could give rise to a variation in the γ -ray flux with a period equal to (or half) the rotation period of the neutron star. We may expect the ultraviolet synchrotron radiation to have a luminosity and periodic variation similar to those of the γ -rays. The timescale for γ -ray production by scattering of stellar photons, $t'_{\text{IC}} \sim 10^3$ s $\gg t_{\text{IC}}$.

Taking the binary separation $a \sim 2R_1 \sim 7 \times 10^{11}$ cm, a rough estimate of the orbital period is $P_{\text{orb}} \sim 1.5$ d. Our estimate of the radial velocity of γ Gem would be $\sim 70 \sin i$ km s⁻¹, where i is the orbital inclination. To be consistent with radial velocity observations^{6,7,11}, we may require i to be small. This would also tend to make detection of the doppler shift of the γ -ray pulse difficult. We note that when γ Gem has evolved to fill its Roche lobe and starts to transfer material at a higher rate, the neutron star will turn on as an X-ray source with properties very similar to those of Her X-1 (which consists of a neutron star orbiting an F subgiant with an orbital period of 1.7 d).

We thank M. J. Rees for useful discussion. J.E.P. acknowledges financial support from I.B.M. A.C.F. acknowledges financial support from the Radcliffe Trust. R.E.D. acknowledges an SRC studentship.

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Evidence that cosmic γ -ray bursts are galactic

OBSERVATIONS from our latest balloon flight and a burst observed by Nishimura *et al.* combined with our previous results and the observations of Bewick *et al.* provide strong evidence that cosmic γ -ray bursts are galactic. It also seems that the recently discovered X-ray bursts and γ -ray bursts have a common origin. A further 41 h of balloon flight time with the University of California, Riverside (UCR) double Compton scatter γ -ray telescope gave no additional γ -ray bursts. This flight time is added to the earlier 24 h observation of Herzo *et al.*¹ who found three bursts with total energy greater than 1×10^{-6} erg cm⁻² and one burst with total energy greater than 4×10^{-6} erg cm⁻² to give lower values for the number of bursts of

$$\left(8 \begin{smallmatrix} +8 \\ -3 \end{smallmatrix}\right) \times 10^2 \text{ and } \left(3 \begin{smallmatrix} +7 \\ -1 \end{smallmatrix}\right) \times 10^2 \text{ d}^{-1}, \text{ respectively.}$$

Our new observations were carried out on a flight launched from Muskogee, Oklahoma on 24 May 1976 at 0100 UT. The altitude of the observations varied from 3.1 to 7.5 g cm⁻² of residual atmosphere. The γ -ray telescope is described elsewhere^{2,3}. The γ rays were detected in three different modes: first, total neutral counts in the large upper liquid scintillator tank (S1) that is $1 \text{ m} \times 1 \text{ m} \times 12.5 \text{ cm}$; and second, total neutral coincidences between S1 and the lower scintillator tank (S2), $1 \text{ m} \times 1 \text{ m} \times 20 \text{ cm}$. Third, γ rays whose energy and angle were identified by scatters in one of the 28 cells in S1 followed by a scatter in one of the 28 cells in S2. For each of these events the cell identification, energy deposit in S1 and S2 and the time of flight from S1 to S2 were recorded. S1 and S2 were each completely surrounded by plastic scintillator and charged particle events were vetoed with an efficiency of better than 99.99%.

The new UCR cosmic γ -ray burst frequencies are given in Fig. 1 along with other satellite and balloon values. The satellites, Vela, IMP 7 and SAS II report observations mainly for larger bursts of 10^{-5} to 10^{-4} erg cm⁻² because of the small sizes and sensitivities of their detectors. The reported Vela burst frequencies^{4,5} bend over at the lower burst sizes presumably because their trigger mechanism is size dependent. Their burst detection frequency then drops rapidly for the smaller bursts. But, the balloon observations with large areas and high sensitivities are able to observe burst sizes as low as $\sim 10^{-7}$ erg cm⁻². Some balloon observations, limited to times of less than about 24 h, have given upper limits only^{6–8}. The 1σ confidence

upper limit of Carter *et al.*⁸, shown in Fig. 1, falls much lower than the other 2σ upper limits. The 2σ confidence upper limit would be higher than the plotted value by a factor of 3. Cline and Schmidt⁹ argue that it should be plotted at a different position (solid rectangle). However, Carter *et al.*¹⁰ insist that their original position is correct. γ -Ray bursts with energies greater than about 10^{-6} and 10^{-7} erg cm⁻² have been observed over a few days and a few hours, respectively. Bewick *et al.*¹¹ pointed out that their point is compatible with a galactic source distribution. Nishimura *et al.*¹² published information about their burst but its contribution to the burst size distribution is reported here for the first time.

If the sources of the bursts are isotropic, either very close, less than a few hundred pc away, or very far—extragalactic—the burst distributions in size (S) should vary as $S^{-3/2}$. On the other hand, if the bursts are galactic and limited to a disk, they should vary as S^{-1} .

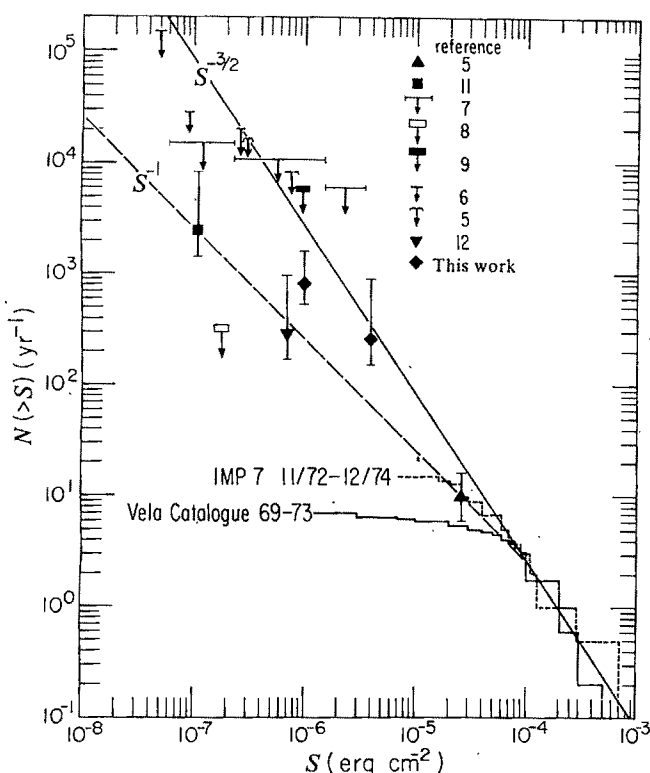


Fig. 1 γ -ray burst frequency distribution. Our points, those of Bewick *et al.*¹¹ and Nishimura *et al.*¹² have error bars of one standard deviation based on a Poisson distribution. The upper limit values are all two standard deviations (95% confidence level) of Gaussian distributions of the backgrounds except for the point of Carter *et al.*⁸ which is 1σ .

The five bursts observed by detectors on balloons along with the observations from satellites at larger burst sizes strongly suggest that the γ -ray bursts are galactic. The larger bursts, seen by satellites, are probably near, within a few hundred pc, and the smaller ones seen by balloons are probably much further away, perhaps thousands of pc.

It is of considerable astrophysical interest to determine whether the recently discovered X-ray bursts¹³ and the γ -ray bursts have a common origin. Their rise times of a fraction of a second and durations of a few seconds seem similar. Although a recent attempt to measure coincidences between the two types of events on the same satellite gave inconclusive results¹⁴, it seems that the evidence supports the same source for the two types of bursts. The γ -ray bursts are then the high energy tails of the low energy X-ray bursts.

We thank the National Scientific Balloon Facility for its balloon launch and NASA, NSF and ONR for their support.

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Statistical investigation of compact emitting regions in extragalactic radio sources

EXTRAGALACTIC radio sources of high total luminosity usually contain compact emitting regions. These may be coincident with the optical galaxy or quasar, or they may be situated at the outer edges of extended double structures. Radio interferometers sensitive to structures with an angular scale ≤ 1 arc s have been used at Jodrell Bank to investigate the frequency of occurrence of such compact regions. Large samples of sources, covering a wide range of flux densities, were studied. This work extends to lower flux densities than previous investigations based on observations of sources showing interplanetary scintillations^{1,2}. The frequency of occurrence of compact regions varies as a function of flux density. This is best explained as a cosmological effect involving the strong evolution of source properties with redshift. Several forms of this evolution, similar to those deduced from the radio source counts (see ref. 3), and the relationship between largest angular size and flux density^{4,5} are discussed elsewhere⁶.

This study is based on observations made with the Jodrell Bank-Defford interferometer (127 km baseline) at 408 MHz using two techniques. The instrument has been used as a drift interferometer to observe sources from the Bologna Catalogue^{7,8} in the declination range $29^\circ 30' - 33^\circ$. A total of 713 sources were observed near meridian transit for approximately 3 min each as they moved through the aerial beams. The fringe visibility (γ_T) in position angle $\approx 180^\circ$ was thereby determined for each source. Although γ_T for any source will depend on its detailed structure, the fraction $p(\gamma_T)$ of sources which have $\gamma_T > 0.5$ indicates the frequency of occurrence of compact regions (≤ 1 arc s) emitting over 50% of the flux density. The results of this survey have been examined as a function of flux density by dividing the sources into five samples which have median values of flux density ranging from 2.95 Jy to 0.45 Jy

Table 1 Drift observations of 713 radio sources from the B2 catalogue^{7,8} at 408 MHz

Median flux density (Jy)	Range of flux density (Jy)	No. of sources	Percentage with $\gamma_T \geq 0.5$
2.95	$2 \leq S < 5$	46	17.4 ± 5.6
1.2	$1 \leq S < 2$	126	19.0 ± 3.5
0.83	$0.7 \leq S < 1$	118	24.0 ± 5.0
0.59	$0.5 \leq S < 0.7$	247	19.4 ± 2.5
0.45	$0.4 \leq S < 0.5$	176	21.0 ± 3.1

as indicated in Table 1; errors of one standard deviation arising from counting errors (and from noise in the two lowest ranges of flux density) are shown. Within the errors, $p(\gamma_T)$ is constant at $\sim 20\%$ over this range at flux density.

Results have been obtained over a much wider flux density range by making tracking observations with the same interferometer. Studies have been made of 385 sources selected without bias from catalogues compiled at 408 MHz. Most of the sources were observed for a 9–12 h period within the hour angle range 1400–0900 h. During such a period the resolving power of this interferometer varies by less than a factor of two, but its position angle changes by more than 120° . The maximum fringe visibility (γ_m) from such observations then gives a better value for the fraction of the flux density coming from regions ≤ 1 arc s as it is largely independent of the effects of any double structure in the source or of elongation of the compact components.

These 385 sources have been divided into nine samples with median values of flux density ranging from 29 Jy to 0.047 Jy as indicated in Table 2. Far more information is available about the structures of intense sources than those of the fainter ones, but in order to search for any trend of compactness with flux density it is desirable to use a statistic which can be extracted, with equal reliability and objectivity, for all the sources being studied. The quantity $p(\gamma_m)$ (fraction of sample with $\gamma_m > 0.5$) is suitable and is plotted in Fig. 1. The values of $p(\gamma_m)$ increase from $11.5\% \pm 8\%$ for the most intense sources to $39\% \pm 10\%$ at 1.6 Jy, but then at lower fluxes remain constant at $\sim 30\%$ or possibly show a slow decrease. For the range of flux densities where the results for the drift and tracking

from 0.12 at 20 Jy (408 MHz) to 0.4 at 2.7 Jy (408 MHz) (the flux densities being converted to 408 MHz using a spectral index of -0.7). These results are clearly consistent with our observations above 1 Jy.

A detailed discussion of the present results will be published elsewhere⁶. Using a standard cosmological model and available data on a sample of sources from the 3CR catalogue as a basis for calculation, it is found that the observed relation, an increase in $p(\gamma_m)$ down to 1 Jy, and a constant value or decrease at lower fluxes, is difficult to explain unless there is a strong evolution of source properties with red shift. The calculations give the best agreement with the observations when a strong evolution of source density³ is coupled with a linear size evolution⁶ of the type $l \propto l_0 (1+z)^{-1}$. Linear size evolution has previously been used to explain the observed form of the largest angular size/red shift relation for QSOs¹⁷ and the largest angular size/flux density relation for radio sources in general⁵. This work suggests that it occurs within the compact emitting regions as well.

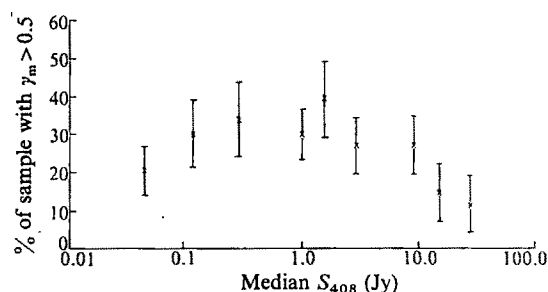


Fig. 1 The fraction of radio sources observed to emit more than half their flux from regions smaller than 1 arc s, as a function of flux density. Errors of 1σ are shown; these are determined from the numbers of sources in the samples and the distribution of individual values of γ_m within each sample.

Further interferometric observations of weak radio sources are in progress in an attempt to reduce the errors in Fig. 1 for the points below 1 Jy.

We thank many colleagues for help with these observations, and H. Darrah, J. Clarke and T. Muxlow for allowing us to use unpublished data. P. J. R., B. S. and R. S. W. all acknowledge support from an SRC maintenance grant during some part of the above observations.

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Received 16 August; accepted 15 December 1977.

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Table 2 Tracking observations of 385 radio sources at 408 MHz

Median flux density (Jy)	Range of flux density (Jy)	No. of sources	% with $\gamma_m \leq 0.5$	Catalogues and observers of compact emitting regions
29	> 20	17	11.5 ± 8	3CR ⁹ , Bridle ¹⁰ , obs. ref. 11.
15	$12.2 < S < 20$	20	15 ± 8.5	
9.5	$8.0 < S < 12$	43	27 ± 8	
3.0	$2.5 < S < 4.5$	45	27 ± 7.5	B2 ^{11,8} , obs. ref. 12
1.6	$1.3 < S < 2.5$	33	39 ± 10	
1.0	$0.90 < S < 1.3$	58	33 ± 7	B2 ^{7,8} , obs. ref. 13
0.30	$0.20 < S < 0.90$	42	34 ± 10	
0.12	$0.090 < S < 0.20$	38	30 ± 9	5C2 ¹⁴ , 5C3 ¹⁵ , obs. ref. 16
0.047	$0.023 < S < 0.09$	89	21 ± 6.5	

observations can be compared, the values of p are consistent. It is found that $p(\gamma_m) \simeq 1.64 p(\gamma_T)$, showing that, for these compact regions, the effects of double structure and component elongation are appreciable but not overwhelming.

Swarup and Bhandari² have investigated the average compactness of radio source components as a function of flux density, using observations of interplanetary scintillation at 81.5, 327 and 430 MHz. They found that the median value of scintillation visibility (a parameter comparable to γ_m) increased

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Carbon and M-type giant stars in the Magellanic Clouds

THE late M- and C-type giant stars are of special interest in studies of stellar and galactic evolution as they are found at the extreme low temperature limit which stars first reach after evolving away from the main sequence, and also because they show remarkably different galactic distribution. Because of the pronounced strength of absorption bands of molecular oxides and of the carbon and cyanogen molecules in the spectra of these stars, extremely small dispersions can be used. Modern large telescopes equipped with Ritchey-Cretien optics allow relatively large fields to be surveyed to faint limiting magnitudes. Thus stars can be segregated and classified whose distance moduli are 22 mag and more. Now stellar populations can be sampled and surveys made of the distribution of the stars in space in the Magellanic Clouds and in the less obscured regions near the galactic centre. Three such surveys are described here. They indicate that unsuspected differences in the mixture of C and late M giant stars exist in the nuclear bulge of the galaxy and in various regions of the Magellanic Clouds. Such studies also yield new information concerning the intrinsic luminosities of C and M giant stars in these three galactic systems.

Nassau and Van Albada¹ showed in 1949 how red giant stars could be recognised and classified spectroscopically in the near-infrared region with a dispersion of $1,700 \text{ \AA mm}^{-1}$ at the atmospheric A band. Their findings were used to determine the galactic distribution of C- and M-type giants. While these two spectral types show strong concentration towards the galactic equator, the M-giants are found in large numbers toward the galactic nucleus and the C stars towards the anticentre². This suggests that within the galactic nuclear bulge a large concentration of M giants must exist and but few carbon stars.

We have, with Hoag's unpublished data, surveyed the clear nuclear bulge region near the globular cluster

NGC6522 known as Baade's Window. The survey was carried out at the prime focus of the Cerro Tololo Inter-American Observatory's 4-m telescope with the aid of a grating-prism combination of the kind described by Bowen and Vaughan³, and by Hoag⁴. A spectral dispersion of $2,300 \text{ \AA mm}^{-1}$ at the A band was achieved. On hypersensitised Kodak IV-N plates, exposed for 60 min, spectra as faint in the near infrared as $m_1 \text{ mag} = 18.5$ in the photometric system of Kron, Gascoigne and White⁵ were recorded; this limiting magnitude exceeds by three magnitudes the faintest red giant star found in the survey. The M giants found are so numerous that if one counts only stars of type M6 or later, one reaches a surface density of 2,400 stars per deg^2 . By contrast, C stars are virtually non-existent in the galactic bulge since only one such star appeared in the search area whose diameter is 23 arc min.

In the present investigation, the same observational instrumentation and techniques were used to study the distribution of red giant stars in the large and small Magellanic Clouds (LMC and SMC). In the LMC seven sample 0.12 deg^2 areas were investigated. Of these, three are found within the LMC Bar, including one in the optical centre, one in the so-called 'radio' centre which marks the centre of the LMC neutral hydrogen distribution and one in the western sector of the Bar; the remainder are at various outlying points up to 3.5° away from the optical centre in the northern, northeastern, eastern and southern directions. For the positions of the optical and radio centres, the coordinates published by Bok⁶ were used. The SMC centres will be described later.

The LMC survey is an extension to a fainter apparent magnitude of the earlier survey carried out by Blanco and McCarthy⁷, who used a thin objective prism with the Curtis Schmidt telescope at Cerro Tololo to study the distribution of late M giants over an 8.75 deg^2 area of the LMC. The spectroscopic dispersion of $6,700 \text{ \AA mm}^{-1}$ in that study allowed the classification of late M giants into a natural group of stars of about M6.5 and later. In the present survey, it was possible to classify each M star to the nearest half subclass. When stars of type M6.5 and later are segregated they show surface densities that are somewhat higher but still in reasonable agreement with the ones found previously; the differences are probably caused by a different cut-off in spectral types near class M6.5, a problem caused by the extremely small spectroscopic dispersion of the thin-prism survey. As will be discussed later, many C type stars are found in the LMC. These were not detected in the

Table 1 Carbon and late type M Stars in selected regions of the Small and Large Magellanic Clouds and in a central region of the Galaxy

Region	Position		C stars	Number		C/M
	R.A. (1975)	Dec. (1975)		M stars		
Small Magellanic Cloud						
SMC central regions						
Bar region	0 h 49.5 min	−73° 25′	80	3		26.7
Wing region*	1 h 00.0 min	−73° 10′	55	1		55.0
SMC peripheral region						
NGC121 region	0 h 36.6 min	−71° 55′	4	4		1.0
Large Magellanic Cloud						
LMC central regions						
O region	5 h 24.5 min	−69° 48′	79	37		2.1
R region	5 h 20.2 min	−68° 55′	38	23		1.7
Bar-west region*	5 h 08.9 min	−69° 06′	71	40		1.8
LMC peripheral regions						
N region	5 h 27.0 min	−66° 24′	7	6		1.2
NE region	5 h 47.0 min	−67° 42′	8	6		1.3
E region	5 h 47.0 min	−70° 30′	32	22		1.5
S region	5 h 24.0 min	−72° 30′	5	12		0.4
Galactic nuclear bulge region						
Baade's Window*	18 h 02.0 min	−30° 02′	1	310		0.003

Area of each region in Table 1 is 0.12 deg^2 .

*Photoelectric sequence has been established in this region.

previous survey although, *a posteriori*, it is possible to recognise them on the thin-prism plates and, in fact, a small fraction (less than 5%) of such C stars were erroneously included in the natural group of late type M giants. Again this is not surprising considering the small dispersion used. An important result of the survey described here is that it confirms the main features of the LMC M-giant distribution by surface density and by brightness found by Blanco and McCarthy, namely, the late M giants are more frequently found in the central regions of the LMC than in the peripheral ones and their magnitude distribution shows a well defined frequency maximum at $m_1 = 14.0$ with a dispersion of ± 0.6 mag. In interpreting their result, Blanco and McCarthy assumed that the late M giants in the solar neighbourhood have an absolute visual magnitude of about -1.0 which, with known $(V-I)$ colours, represents a near infrared luminosity $m_1 = -4.6$, similar to that found for the LMC M giants. However, the previous survey made in Baade's Window suggests strongly that the galactic disk late type giants are actually two or more magnitudes fainter than $M_v = -1.0$. Thus there is a marked luminosity difference between M giants in the LMC and in the galactic nuclear bulge. LMC giants of type M seem to be quite comparable to the red giants found in globular clusters in the Galaxy. Although this result is consistent with early theoretical expectations of Hoyle and Schwarzschild⁸, the precise luminosity to be expected of the red M giants in the LMC cannot now be estimated because we lack precise knowledge of metal abundances of late type stars even in the nuclear bulge of our own galaxy. Now we are for the first time detecting the giant branch of late type stars in nearby galaxies. Abundance data must come later from photometric and higher dispersion spectral surveys in the future.

Three SMC sample areas were also included in the current investigation. One is located in the SMC Bar, another in the Wing and the third in an outlying region near the cluster NGC 121. Table 1 shows the results of the present survey for both Magellanic Clouds. By way of comparison our previous results for Baade's Window are also listed. While C stars are extremely rare in the galactic nuclear bulge, they are found frequently in the Magellanic Clouds especially in the central regions. Ordered according to decreasing metallicity, these regions would be as follows: galactic nuclear bulge, LMC, and SMC. Table 1 reflects the same ordering in the ratio of the number of C stars to the number of late M giant stars but in a reversed sense: almost no C stars are seen in the nuclear bulge where late type M giants abound; C stars outnumber M stars about 2 : 1 in the LMC central areas, while in the SMC practically no M stars are found and the C stars are most frequent in occurrence.

From previous knowledge, the peripheral regions of our galaxy could also be included in this ranking somewhere between the nuclear bulge and the LMC. Although this suggests that chemical composition may be an important determinant of the ratio of C to M giants, age differences must also be taken into account. Some C and M type giant stars have been found⁹ in globular clusters in the Clouds but their number is too small to allow one to make reliable correlations with the metal abundances of the parent globular clusters. Improved theoretical knowledge of cool giant stars will be required before these results can be better interpreted. Nevertheless the present study indicates that if having a C type spectrum is but a stage in the evolution of red giants, as suggested by Wallerstein¹⁰, then the galactic bulge M giants, numerous as they are, must all be approximately coeval and have similar masses and chemical compositions.

Near infrared (m_1) photometry of the C stars was done in the present study, using Kodak I-N plates exposed through a Schott RG 695 filter and newly established photoelectric

near-infrared magnitude sequences. The mean m_1 value for the LMC C stars is 14.0 for the field which contains the photoelectric sequence, LMC Bar-west; for the SMC the corresponding value is 14.6 m_1 (in the SMC Wing region which also has a photoelectrically determined magnitude sequence). The difference is that to be expected from the distance moduli of the two Magellanic Clouds. In both clouds, then, the near infrared luminosities for C stars is $M_1 = -4.6$, similar to the M giants as found above. Earlier reliable estimates of the luminosities of C stars are lacking, but Gordon's study¹¹ suggests that no marked difference exists with the luminosity of the carbon stars in the solar neighbourhood.

The frequency of C stars in the central or bar regions of the two clouds is remarkably high, about 600 stars per deg.². This is another indicator that the clouds have had rather different evolutionary histories from the galaxy.

Comparing the Table 1 data for the LMC and SMC late type giants one finds the SMC extremely deficient in late type M giants. Also, stars as late as M9 are found in the direction of the galactic centre, stars as late as M8 are found in the LMC but in the SMC no M star later than M6 has been found. Detailed lists of the stars found in this study including photometric and positional data will be published elsewhere.

Earlier studies of the red giants in the LMC but to a much brighter limiting magnitude were made by Westerlund and coworkers^{12,13}; these did not apparently detect the main features of the distribution of red giants outlined here, although undoubtedly the very brightest of the C stars and the M supergiants were recognised and recorded. More recently Sanduleak and Davis Philip¹⁴ have surveyed the C stars in the SMC with objective prism spectra obtained in the spectral region of the C₂ Swan band using the Curtis Schmidt telescope. The overall distribution found by them seems to be correct but their survey shows only a small fraction of the stars detected in the current investigation. Sanduleak provided us with a list of the positions of his SMC carbon stars so that we could compare results.

We thank the USNSF for the operation of the Cerro Tololo Inter-American Observatory. M.F.M. thanks the Foundation for support during a stay in Chile as visiting scientist at CTIO. We also thank Dr P. J. Treanor, Director of the Vatican Observatory who enabled us to meet and analyse our observations, and finally, Dr A. Hoag, Director of Lowell Observatory, who helped us to develop the observational technique used in this study.

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Received 24 October; accepted 6 December 1977.

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How to locate the electrically conducting fluid core of a planet from external magnetic observations

A NEW method is proposed here for locating the electrically conducting fluid core of a planet from external magnetic observations. When tested with the aid of geomagnetic data, the method gives for the radius of the Earth's liquid metallic core a value which differs insignificantly from the accepted value (based on seismic data). These findings have important implications for future investigations of the magnetic fields and internal structure and dynamics of the Earth, Jupiter, and other magnetic planets.

Consider, as a good first approximation to a real planet in the present context, an isolated spherical planet of radius r_s consisting of an electrically conducting spherical fluid core of conductivity σ and radius r_c surrounded by a non-conducting mantle of thickness $r_s - r_c$. Suppose that the planet is embedded in a non-conducting atmosphere which extends to infinity and that the whole of space $r > 0$ is pervaded by a magnetic field $\mathbf{B}(\mathbf{r}, t)$ due entirely to electric currents of density \mathbf{j} flowing in the conducting core. Any effects due to permanent magnetism are assumed negligible so that the magnetic permeability μ is everywhere equal to that of free space. (Here t denotes time and \mathbf{r} is the vector distance of a general point from the centre of the planet.)

Specifying \mathbf{j} in $0 < r < r_c$ suffices to determine \mathbf{B} throughout the whole of the planet and the surrounding space, but the inverse problem of determining \mathbf{j} in $0 < r < r_c$ from a full description of \mathbf{B} outside the core has no unique solution. Nevertheless, as we shall argue later, measurements at any epoch t of $\mathbf{B}(\mathbf{r}, t)$ and also of its time rate of change $\partial\mathbf{B}/\partial t$ over any closed surface that encloses the planet can be used to determine the radius r_c of the core of the planet in a simple and straightforward way. This is so provided that measurable fluctuations in \mathbf{B} occur on time scales τ that are so short in comparison with the time scales on which effects due to ohmic decay make themselves felt that the fluctuations can, with sufficient accuracy, be ascribed largely to the rearrangement of lines of magnetic force by fluid motions in the core.

The last condition requires that

$$\tau \equiv \overline{B/\partial B/\partial t} < \mu \sigma \alpha^2 r_c^2 \quad (1)$$

where \overline{B} and $\overline{\partial B/\partial t}$ are typical magnitudes of \mathbf{B} and $\partial\mathbf{B}/\partial t$, respectively, and αr_c is a length-scale characteristic of the spatial distribution of \mathbf{j} in $r < r_c$, α being typically ≤ 1 . Over time intervals satisfying equation (1) (which are, of course, very much less than the time scale associated with the generation of the magnetic field by the dynamo process) we can, to a first approximation, treat the core as a perfect electrical conductor, for which it is impossible, by Faraday's law of electromagnetic induction, to change the magnetic flux linkage $N(r = r_c)$ (ref. 1, for further details and references see refs 2 and 3). (Fluctuations in $N(r = r_c)$ when σ is infinite would generate electric currents of infinite strength, requiring an infinitely powerful source of energy.) For any fixed closed (but not necessarily spherical) surface Σ given by $r = R$ which encloses the origin $r = 0$ we can define a quantity $N(t; r = R)$ equal to the total number of intersections of lines of magnetic force with Σ ; thus

$$N(t; r = R) \equiv \iint_{\Sigma} |\mathbf{B} \cdot d\mathbf{A}| \quad (2)$$

$d\mathbf{A}$ denotes the vector element of area of Σ , over the whole of which the area-integral is taken. At the bounding surface of a perfect conductor, N is independent of t , so that $\delta N/\delta t$ vanishes and so do all higher derivatives $\delta^2 N/\delta t^2$ and so on.

The quantity N can be calculated quite simply (in principle) for all surfaces Σ that lie outside the core, as $\mathbf{j} = 0$ in $r > r_c$ and \mathbf{B} in $r > r_c$ can therefore be expressed as the gradient of a scalar V (say) satisfying Laplace's equation $\nabla^2 V = 0$. This property enables \mathbf{B} to be determined everywhere in $r > r_c$ from measurements of \mathbf{B} over any single closed surface in that region, such as the accessible surface of the planet in $r = r_s$ or a closed surface effectively covered by an orbiting satellite carrying a magnetometer. Thus, this new method for determining the radius r_c of the electrically conducting core of a planet from measurements of \mathbf{B} in $r \geq r_s$ involves, in principle, taking such measurements for two epochs $t = t_1$ and $t = t_2$ separated by a time interval $t_2 - t_1$ satisfying the inequality expressed by equation (1), and calculating from these measurements the quantity

$$\Delta N(t_1, t_2; r = r_s - p \Delta r) \equiv N(t_2; r = r_s - p \Delta r) - N(t_1; r = r_s - p \Delta r) \quad (3)$$

at successive levels $r = r_s - p \Delta r$ within the planet, where $p = 0, 1, 2, 3, 4$, and so on, marching downwards from the surface in conveniently short steps of length Δr . In general, ΔN will be non-zero when $p = 0$ (corresponding to the surface of the planet $r = r_s$) and also at successive values of p , if Δr is small enough, until p reaches that value p_c (say), at which ΔN effectively vanishes. The value of r_c is then given by

$$r_c = r_s - p_c \Delta r \quad (4)$$

To avoid ambiguity, it is necessary to find all values of p at which ΔN is equal to zero and then select the correct value of p_c by requiring also that higher time-derivatives of N should vanish.

Further details of the method, including the systematic analysis of the physical and mathematical approximations upon which it is based, and the treatment of errors will be reported elsewhere (R.H., F. J. Lowes & S. R. C. Malin, in preparation). It is enough to remark here that the first practical result based on the new method is encouraging (if it is not fortuitous), for when applied to the Earth, for which $r_s = 6,371$ km, the method gives for r_c a value which differs insignificantly, by less than 2%, from the accepted value $3,486 \pm 5$ based on seismological data. (This calculation was kindly carried out by S. R. C. Malin and colleagues at the Geomagnetism Unit of the Institute of Geological Sciences, NERC, using one of the best available models of the geomagnetic secular variation, where determinations of the main geomagnetic field—namely that field obtained when contributions due to ionospheric and magnetospheric currents have been removed by taking annual mean values of the observations—for the period 1965 to 1975 were fitted by a spherical harmonic series up to and including terms of degree 8.) The method should prove valuable in the investigation of the internal structure of other magnetic planets, such as Jupiter⁴, Saturn and Mercury (for which the sizes of the electrically conducting fluid cores are not yet known), provided that sufficiently detailed measurements of their main magnetic fields and secular changes can be made in future work with space-probes. If the pressure at the level $r = r_c$ in Jupiter is equal to the critical pressure P at which molecular hydrogen changes to its metallic form, which is several megabars and well above the range of operation of high pressure laboratory apparatus, then the determination of r_c by the proposed new method will lead directly to an estimate of P which would serve as a check on estimates⁴ based on shock wave experiments and quantum mechanical calculations. On the other hand, if r_c is so close to r_s that the corresponding pressure at $r = r_c$ is well below present estimates of P (even allowing for the large uncertainties in these estimates, more than a factor of two), then it would be necessary to take seriously the possibility (see refs 4 and 5) that impurities render the molecular hydrogen layer in Jupiter sufficiently conducting to support dynamo action within it.

I thank Dr C. T. Russell for correspondence and a preprint⁷ that prompted the publication of this note.

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Received 11 November; accepted 21 December 1977.

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ULF tree potentials and geomagnetic pulsations

HIGH-SENSITIVITY measurements of ultra-low-frequency (ULF; frequencies less than 5 Hz) geomagnetic pulsations¹ usually require elaborate receiving antennas ranging from large air-cored coils² through multi-turn steel, or mumetal-cored solenoids^{3–5} to small superconducting loops immersed in liquid helium⁶. Pairs of electrodes inserted in the ground have also been used as antennas^{7,8}. The need for a large spacing between the electrodes (varying from hundreds to thousands of metres) and the difficulty of calibrating the measurements absolutely have resulted in the almost universal use of the more compact and easily calibrated coil-type antennas in recent years. I describe here a new method for measuring ULF geomagnetic pulsations, which requires a minimum of elaborate equipment. The method is based on the use of trees, or, more specifically, on the use of pairs of electrodes inserted into trees, as ULF receiving antennas.

There are several reasons that this new method of measurement may be of interest. The equipment is simple and thus the method could lead to more widespread observations of ULF geomagnetic pulsation phenomena. The method of measurement also provides new information about tree potentials, that is, it shows that some, and perhaps all, of the ULF components of these potentials are induced by ULF geomagnetic field fluctuations and do not originate in the trees themselves. Finally, although it is not clear at present what effect induced ULF electric fields may have on the growth and other vital processes in a tree, the link between these ULF electric fields and geomagnetic field fluctuations suggests that some environment-related changes in trees could also be influenced by changes in geomagnetic activity. These changes may have a natural origin (for example, the changes that occur during a solar cycle⁹) or they may be caused by a variety of human activities (by modern d.c.-powered mass transit systems, which can produce large amplitude ULF electromagnetic fields¹⁰).

The ULF measurements reported here were stimulated by the work of Burr on relatively steady-state tree potentials¹¹. Burr recorded these potentials for more than a decade using a pair of specially-designed non-polarisable electrodes inserted in the cambium of an unspecified tree (which was probably a maple). The electrodes were about a metre apart along the long axis of the tree and Burr observed diurnal, 27-d, and seasonal variations, as well as a suggestion of a correlation with sunspot activity, in their potential difference.

Most of Burr's observations were at frequencies far below the frequency range for ULF geomagnetic pulsations. One series of measurement obtained, however, during an electrical storm suggested that ULF variations of tree potentials might occur on occasion. I therefore began a search for variations with frequencies predominantly in the

Pc 1 geomagnetic pulsation range (0.2–5 Hz). These frequencies correspond approximately to the delta regime for human brain waves.

The measurements were made using a large native oak, *Quercus lobata*, that was located near conventional ULF recording equipment at a site on the Stanford University campus. This latter equipment uses 20,000 turn steel-cored solenoids as ULF antennas and it operated continuously throughout the interval during which the tree measurements were made. Thus, simultaneous measurements of ULF geomagnetic pulsations using both conventional loop antennas and a tree 'antenna' were obtained at the one location.

Two steel nails were used as electrodes. Following Burr's configuration, they were inserted about 0.05 m into the tree along the long axis, with a spacing of 0.76 m. The lower electrode was approximately 1 m above the ground, and the two electrodes faced toward the geomagnetic west. Because the tree was not completely vertical, a line joining the two electrodes would have been inclined approximately 20° toward the geomagnetic east. The diameter of the tree midway between the two electrodes was 0.65 m.

A resistance of about 5 k Ω was typically observed between the electrodes, increasing to about 10 k Ω if polarisation was allowed to occur. A d.c. potential difference was also observed that varied from day to day but whose absolute value was usually in the range 10 to 100 mV, with the upper electrode positive. The electrodes were connected to a low-frequency high-gain amplifier through an RC filter ($R=22\text{ M}\Omega$, $C=50\text{ }\mu\text{F}$). The amplifier was usually set for 50 db gain, and its output was filtered (0.02–7 Hz) before being recorded, generally without additional amplification, on a chart record and on analog magnetic tape.

The ULF signals measured by this system were undoubtedly induced in the tree 'antenna' and not in the shielded cabling between the electrodes and the recording system: when the electrodes were disconnected from the tree and connected to an equivalent 5 k Ω resistor, without any other change in the wiring or configuration of the system, only a steady low level of white noise (typical resistor thermal noise) was observed.

Similarities between the ULF signals recorded conventionally and with the tree 'antenna' were immediately apparent on the chart records. More detailed analysis confirmed that Pc 1 pulsation events recorded by the two systems were very nearly identical in all their important characteristics. Figure 1, for example, shows spectrograms of a sequence of four Pc 1 pulsation events that occurred during the interval 1200 to 1500 UT on 17 January 1976, and which were received by the tree 'antenna' (a) and the conventional north-south solenoid antenna (b). With the exception of a lower signal-to-noise ratio for the tree measurements, the two Pc 1 pulsation records are closely alike. It will also be noticed that the lower frequency Pc 2/Pc 3 geomagnetic activity (frequencies in the range 0.02 to 0.2 Hz) is recorded similarly by both systems. The amplitude of the ULF pulsations in the tree potentials is very small. For the Pc 1 pulsations shown in Fig. 1, the maximum amplitude of the potential fluctuations was about 0.1 mV.

The nearly identical occurrence and spectral characteristics of ULF events measured by the tree electrodes and by the conventional ULF equipment indicated that the tree potentials were largely induced by ULF time variations of the geomagnetic field. To investigate this possibility, a portable planar search coil powered by a 1 Hz signal generator was moved around the tree near the electrodes. It was found that a 1 Hz oscillation of the potential difference between the tree electrodes was produced only when the search coil was orientated with its moment vector in the north-south direction. When the two electrodes were moved to the north face of the tree, a response from the electrodes could be obtained only when the search coil moment vector was orientated in the east-west direction.

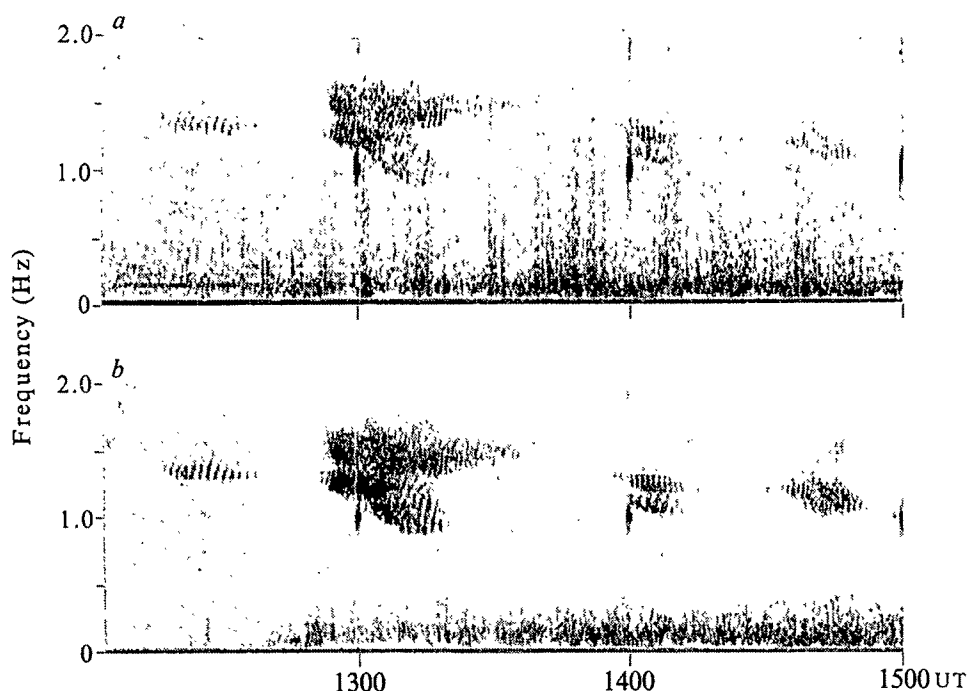


Fig. 1 Spectrograms of a series of Pc 1 geomagnetic pulsation events recorded at Stanford, California, using tree potentials (a) and a conventional solenoid antenna (b). Short intervals of a 1 Hz calibration signal appear at the start of each hour. The vertical lines in the upper spectrogram are caused either by local electromagnetic transients or by natural sferics; similar lines occur in the lower spectrogram, but they are not as obvious because the background noise is comparatively suppressed.

These results, and the observations of natural Pc 1 pulsations, can possibly be best understood by considering the tree/electrode pair combination to form a collection of conducting loop antennas in which e.m.f.s may be induced by magnetic field fluctuations in the appropriate direction. The conducting paths are provided by the conducting material of the tree (and the cambium in particular¹¹), and, for field fluctuations in a particular direction, the area of the relevant loop antenna is defined by the intersection of the tree with a vertical plane perpendicular to the particular field direction and passing through the two electrodes. Thus, in the measurements reported here, the Pc 1 pulsation events observed in the tree potentials were produced by Pc 1 pulsations of the north-south component of the geomagnetic field.

Further tests showed that the tree potentials could only be detected in a living tree. Thus, when a tree dies, the potentials gradually disappear as the wood dries and loses its conductivity.

In conclusion, measurements with tree electrodes show that trees may be used as 'antennas' to detect ULF geomagnetic pulsations. The measurements also show that ULF tree potentials are largely produced by ULF fluctuations of the geomagnetic field (the remaining component of the potentials is probably thermal noise). Presman¹² noted that electromagnetic fields usually have an adverse effect on living processes. If the ULF geomagnetic pulsations have any adverse effect on the growth of trees (and, as we have seen, they must induce electric currents in the living material) these effects could possibly be observed in tree ring data. Pc 1 geomagnetic pulsation occurrences vary markedly over a solar cycle⁹ and thus, if these particular pulsations affect tree growth, a solar cycle in tree ring data could occur. LaMarche and Fritts¹³ searched unsuccessfully for a relation between tree ring data and sunspot numbers. The phase of the Pc 1 pulsation solar cycle, however, differs by several years from the sunspot cycle and, assuming the two cycles affect tree ring data, they may tend to obscure each other's effects. Furthermore, other geomagnetic pulsations and higher-frequency electromagnetic signals have their own cycles of occurrence, and their effects on tree ring formation, if any, could add further to the complexity of the tree ring data. Studies of these possible effects are desirable, because the tree ring data could provide a unique

record of past ULF and higher-frequency geomagnetic activity.

I thank D. B. Coates for technical assistance. This work was supported in part by the Advanced Research Projects Agency of the US Department of Defence and in part by the US Office of Naval Research.

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Mass spectrometric measurement of the positive ion composition in the stratosphere

THE ion composition of the upper atmosphere has been the subject of many experiments. Early mass spectrometer measurements have been primarily concerned with the ion composition at altitudes between 60 and 250 km (refs 1-6). Few data, however, are available on the charged particle composition of the stratosphere. Global ion density measurements using electric probes⁷⁻¹¹ indicate number densities of the order of 100-10,000 cm⁻³ for positive ions in this region. Arnold *et al.*¹² have recently published the first mass spectrometric data of the stratospheric ion composition, obtained on the downleg portion of three rocket flights. Above 40 km they observe the proton hydrates as being the most abundant ions. Below 40 km a change in the predominant ion species is seen, which is explained by Arnold *et al.* by ion molecule reactions between water cluster ions and

formaldehyde. One of the possible dangers, inherent to rocket flights, however, is the alteration of the ion composition, due to shock wave-induced fragmentation. Mass spectrometric measurements on this region using a balloon-borne instrument were suggested¹³ as early as 1969. Here we give the first preliminary results of a successful flight, performed with a balloon-borne quadrupole mass spectrometer on 30 September 1977. A 100,000 m³ Zodiac balloon was flown at mid-latitude (CNES launching site at Aire sur l'Adour, France, 44°N) at 18.27 UT and reached the altitude of 35 km at 20.00 UT. The measurements were started at 20.14 UT, which is after sunset.

The gondola, which will be described in more detail elsewhere, primarily consists of a high-speed helium cryopump, in which is built a quadrupole mass filter, followed by a high gain electron multiplier. The ions were sampled through a 0.2 mm hole in a 0.1 mm thickness stainless steel flange, which can be biased with respect to the gondola structure. Signal detection is realised

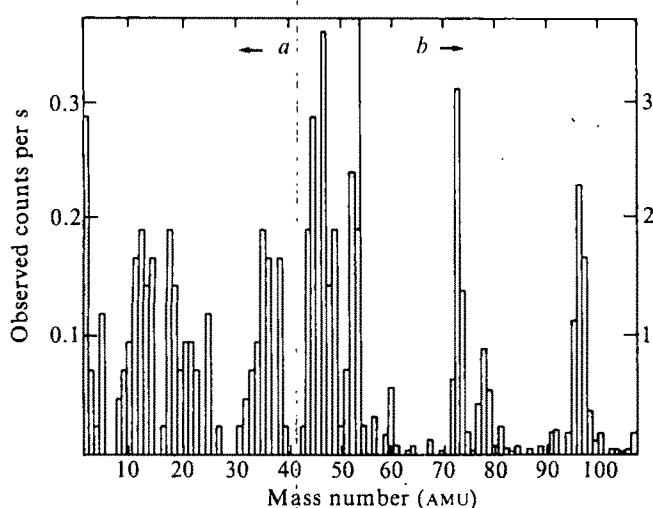


Fig. 1 Observed count rate versus mass number measured at 35 km altitude. Both *a* and *b* have been obtained by averaging of the counts observed, during seven scans. The applied draw-in-potential was -13 V.

by both analogue and pulse counting techniques. The mass spectrometer covers the mass range from 0 to 109 AMU. Measuring in mass domains as well as sweeping the complete mass range is possible¹⁴. Sweeping the complete mass range was realised by using discrete steps approximately 1 AMU in size. The integration time for pulse counting was 6 s per step. Thus spectra were obtained in the form of a histogram, as is shown in Figs 1 and 2. A fixed resolution mode was used throughout all scans reported here. Two separate draw-in-potentials were used, -13 and -4.6 V. Figure 1 represents the results obtained at -13 V and Fig. 2 those at -4.6 V. It should be noted that in both figures the number of observed counts per second for masses smaller than 55 AMU have been represented with a different scale factor. The count rate due to noise pulses was smaller than 0.1 s^{-1} during the whole flight.

The mass numbers of the detected ions are listed in Table 1, with the draw-in-potentials and the uncertainty in atomic mass numbers. Counts below mass 10 have not been taken into account, because instabilities have been observed in the power supply of the quadrupole for such low mass values.

As can be seen from Table 1, the uncertainty for lower mass numbers is rather high for two reasons. First, the fixed resolution, chosen rather low in favour of high sensitivity, gives rise to much more broadened peaks at lower masses, than at higher ones. Second, due to the low counting rate at low mass numbers, it was impossible to build up a complete histogram even after several scans and thus a faulty conclusion may be drawn about

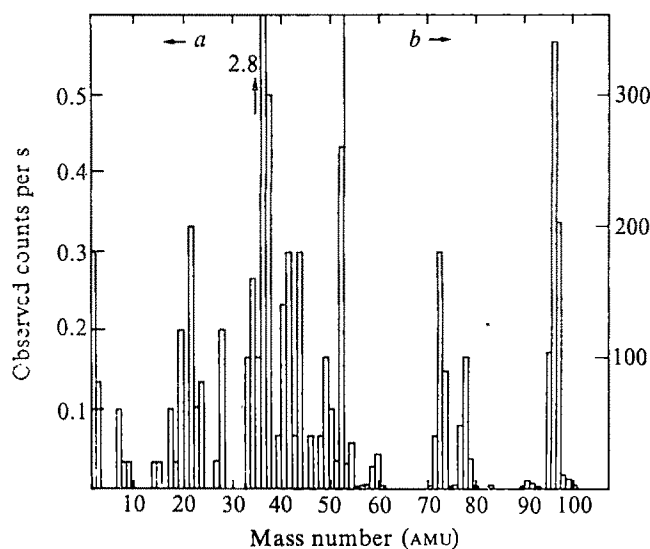


Fig. 2 Observed count rate versus mass number at 35 km altitude. *a*, The result of five scans; *b*, two scans. Applied draw-in-potential was -4.6 V.

the exact position of the pass peaks. Above mass 50, however, the signal was strong enough to permit a higher degree of certainty. Furthermore, the mass domain from 50 to 109 AMU has been scanned with a higher resolution and the results confirm those of Table 1. Masses $19 (18 \pm 3)$ and 20 ± 3 , 37, 55, 73 and 109 can be identified with the cluster ions $\text{H}^+(\text{H}_2\text{O})_n$, n ranging from 1 to 6.

In all spectra, mass 73 was the most abundant peak among the water cluster ion peaks. The observed distribution of the $\text{H}^+(\text{H}_2\text{O})_n$ cluster ion peaks is represented in Table 2.

Table 2 does not represent a correct number density distribution of the hydrated hydronium ions, as a correction factor has to be taken into account to convert peak height to number density (even on relative scale). The result of this correction, which is not known exactly and which cancels the effect of lower transmission of the mass filter at higher mass numbers, will be a larger percentage of mass 91. As possible cluster breaking up due to electric fields must also be considered, our results are difficult to compare with the distribution calculated by Mohnen¹⁵.

It is not completely clear why the distribution of $\text{H}^+(\text{H}_2\text{O})_n$ is different at different draw-in-potentials, although again electric field-induced breaking up of the cluster ions may have an important role.

Apart from the water cluster ions, which are expected according to the known reaction schemes¹⁶, additional peaks

Table 1 Observed mass numbers

Draw-in-potential		
-13 V		-4.6 V
13 ± 3		—
18 ± 3		20 ± 3
25 ± 3		—
—		29 ± 3
37 ± 3		37 ± 2
—		43 ± 3
47 ± 3		50 ± 3
55 ± 2		55 ± 2
60 ± 2		60 ± 2
73 ± 2		73 ± 2
78 ± 2		78 ± 2
91 ± 2		91 ± 2
96 ± 2		96 ± 2
109 ± 2		—

Table 2 Observed distribution of $H^+(H_2O)_n$ mass peaks at 35 km in %

n	Draw-in-potential Mass number	-15 V	-4.6 V
2	37	5	1
3	55	6	16
4	73	79	80
5	91	5	3
6	109	5	—

have been observed, among which 96 ± 2 was the most abundant. At low draw-in-potentials, this peak was even larger than the one at mass 73. Masses 29, 42, 60 and 80 have also been observed by Arnold *et al.*¹², but they do not mention mass 96 ± 2 , nor do they report that they have observed masses 73, 91 and 109. Because this (96 ± 2) is the most abundant mass peak among the non-proton hydrates which we have observed, it is tempting to conclude that rocket-borne measuring devices are more disturbing than balloon-borne instruments. However, care must be taken, as the ion-molecule reaction channels might be different during night time. The observed mass peaks can be partly fitted into the formaldehyde ion chemistry if 47 ± 3 and 50 ± 3 are tentatively identified as $H^+(CH_2O)H_2O$ and 96 ± 2 as $H^+(CH_2O)_2(H_2O)_2$ or $C_2H_2O^+(H_2O)_3$. But, one should be careful because although the existence of formaldehyde in the stratosphere has been predicted theoretically¹⁷, to our knowledge no experimental observation of it has been published. Furthermore, no reaction rate constants of formaldehyde ions or molecules with proton hydrates have been published. Thus any tentative identification must be regarded as speculative. As an alternative interpretation, however, mass numbers 60, 78 and 96 can be tentatively assigned to the hydrates of N_3^+ , namely $N_3^+(H_2O)$, $N_3(H_2O)_2$ and $N_3^+(H_2O)_3$, respectively, which have already been observed in laboratory air flow discharges by Hayhurst and Padley¹⁸. In view of this, 47 ± 3 and 50 ± 3 may be interpreted as due to O_3^+ .

We need more data, especially higher resolution mass spectra and conclusive laboratory work, before any final conclusions can be made. It is clear, however, from the observations reported here and from those of Arnold¹², that apart from the hydrated hydronium ions some other unknown ionic species are present in the stratosphere. This should give a new motivation for more extended laboratory and *in situ* investigations on the ionic chemistry of the stratosphere.

We thank Ir. C. Lippens and the technical staff of the Belgian Institute for Space Aeronomy, who made this experiment possible and Drs M. Ackerman and G. Kockarts for helpful criticisms.

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Received 25 October; accepted 15 December 1977.

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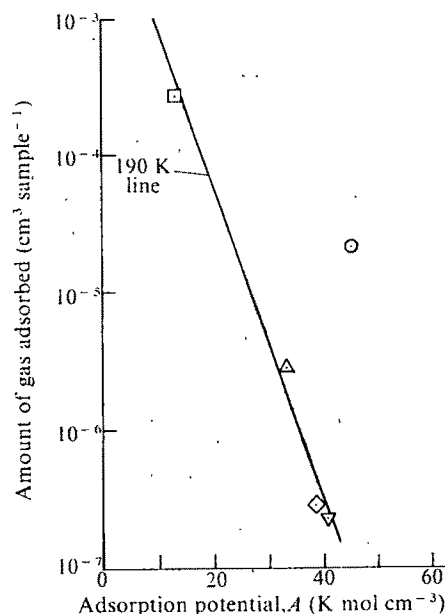
Chemical interpretation of Viking Lander 1 life detection experiment

PRELIMINARY results of the Viking Lander 1 (VL-1) biology experiments¹ revealed that humidification of the martian soil sample in the gas exchange experiment (GEX) released substantial amounts of carbon dioxide and oxygen, as well as detectable amounts of nitrogen and argon or carbon monoxide. We have reviewed the available flight data and found that, when the amounts of evolved gases were plotted in an adsorption potential plot, the amount of evolved oxygen was anomalously high compared to the other gases. This paper also describes simulation experiments with a model Mars soil provided by the Viking Inorganic Analysis Team (ICAT) and treated with a radiofrequency (RF) glow discharge in a simulated martian atmosphere. The findings indicated that the GEX simulation procedure released oxygen, carbon dioxide, and nitrogen in amounts comparable to that seen in the experiment on Mars.

The design of the GEX and its modes of operation have been described elsewhere². The values first reported¹ have been revised, and the maximum amounts of gases evolved from the entire 1 cm³ of humidified sample in the test cell have been given by Oyama *et al.*³ as 83 nmol N₂, 775 nmol O₂, 9,800 nmol CO₂, and 13 nmol Ar/CO. Ar and CO were not separated in the chromatographic column.

The postulate considered here for the gas evolved in the VL-1 humid mode GEX was that the amount of gas seen could have been physically or chemically adsorbed, or attached in a surface complex, on the martian soil. Using a molecular area of 14.6×10^{-20} m² for O₂ and an estimated bulk density of soil sample of 1.3 g cm⁻³, it can be calculated that the evolved O₂ would cover an area of 0.052 m² g⁻¹ soil. The estimated bulk density was the same value as that used in ref. 3 and was the midpoint of the range estimated in ref. 4 from the observed physical behaviour of the fine-grained materials in the immediate vicinity of VL-1. A similar calculation for the CO₂, using a molecular area of 17×10^{-20} m², gives a surface coverage 0.77 m² g⁻¹. A specific surface area of 1 m² g⁻¹ or greater could, therefore, have accommodated the evolved O₂ and CO₂.

Fig. 1 Adsorption potential plot of gas evolved in VL-1 GEX humid mode experiment. The 'A value' ordinate includes sample temperature, T , in K, molar volume of liquid adsorbate, V_m , in cm³ g⁻¹, and the reciprocal of the relative vapour pressure of the adsorbate, P_0/P where $A = (T/V_m) \log_{10} (P_0/P)$. \diamond , CO; \circ , O₂; \square , CO₂; \triangle , N₂; ∇ , Ar.



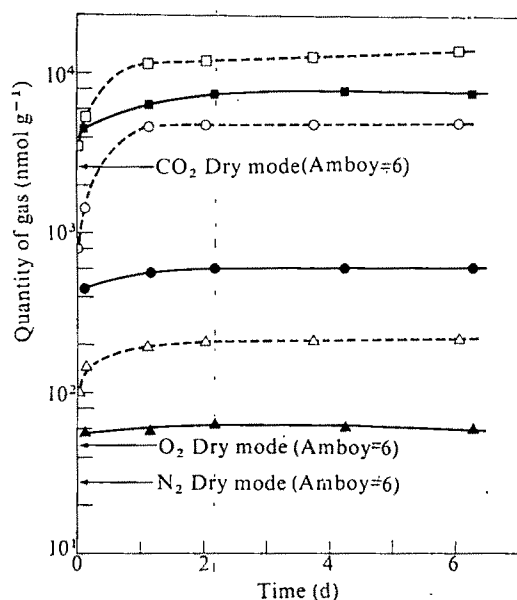


Fig. 2 Gas released in the humid mode from the VL-1 GEX (solid line) and from RF glow discharge treated Amboy no. 6 soil (dashed line). RF glow discharge conditions were 33 mm³ STP s⁻¹ O₂ flow, 0.50 mm Hg = 66 Pa O₂ pressure, 50 W RF power at 13.56 MHz, 398 K sample temperature, and 18 h = 65 ks treatment time (d × 86.4 = ks) ●, ○, O₂; ■, □, CO₂; ▲, △, N₂.

Data for the gas composition of the martian atmosphere⁵ and the equilibrium vapour pressures of the pure gases⁶ were used to calculate the relative pressure, P/P_0 , of the gases in the martian atmosphere at a soil acquisition temperature of 190 K (ref. 7). An adsorption potential plot, similar to that used for the prediction of adsorption of trace gas species in spacecraft contaminant control systems⁸, is shown in Fig. 1. The CO₂, N₂, CO, and Ar points approximate a straight line, but the O₂ point is anomalously high. An adsorbed monolayer volume was derived from the potential plot by extending the straight line to the 'A value' range for physical adsorption of nitrogen at the temperature of boiling liquid nitrogen, and plotting the extrapolated adsorption data as a BET plot. A surface area of 17 m² g⁻¹ was calculated for the martian soil, from a nitrogen molecular area of 16.2 × 10⁻²⁰ m².

A simulated martian soil, Amboy no. 6, had been obtained by ICAT from the Amboy volcanic crater in southern California. It was chosen as a simulated martian soil because of its low organic and high iron content. More recently, synthetic mineral mixtures have been made-up as model martian soils, but it is believed that differences between the Amboy soil and later synthetic models would not be consequential to studies described here. The Amboy no. 6 soil sample was first mixed with 0.01% calcium superoxide to test sample handling and analytical techniques for the GEX simulation. The superoxide and soil were mixed to provide a suitable mass of sample for handling in the simulation apparatus, and the amount of superoxide alone was in the microgram range. The model soil had been ground, sieved, and remixed and its BET surface area was 22 m² g⁻¹. The oven-dried (423 K) sample was subjected to a humid atmosphere in the GEX simulation apparatus and released 1,820 nmol O₂, which was 5% more than calculated from reaction stoichiometry.

Because the sample handling and analytical techniques of the GEX simulation seemed adequate to test the release of small quantities of oxygen in a humid atmosphere, the release of oxygen from labile oxygen-containing surface complexes was tested next. A 1 g sample of Amboy no. 6 soil was pretreated to remove adsorbed water and equilibrated with a simulated martian atmosphere. The simulated martian atmosphere was

made and analysed by Matheson Gas, as 2.50 volume % N₂, 0.33 volume % O₂, 95.66 volume % CO₂, 1.35 volume % Ar, and 0.15 volume % CO. The moisture content of the mixture was 48 p.p.m. The Amboy no. 6 soil sample was subjected to a RF glow discharge in conditions chosen to expose the sample to active oxygen species at a high enough concentration level to accelerate the kinds of reactions possible between a soil and the naturally occurring active oxygen species at the surface of Mars⁹. A control sample was carried through the same time, temperature, and oxygen flow regime in the reactor, but without the RF field.

Figure 2 shows results obtained from the GEX humid mode simulation, and comparable results from VL-1 GEX humid mode data. The relative amounts of evolved gas species and their rates of evolution were similar for the GEX data and the simulation experiment, suggesting that the GEX oxygen release could have been the consequence of the long time of interaction of the martian soil with reactive oxygen species produced in the atmosphere by ultraviolet radiation.

The treatment of the VL-1 GEX humid mode data, together with the results of the simulation experiment, leads to several conclusions. First, the quantities of gases released at the temperature and humidity conditions of the GEX test cell were in agreement with a model of physical adsorption from the martian atmosphere on a soil of 17 m² g⁻¹ surface area, except for the anomalously large quantity of oxygen—which must be accounted for by oxygen adsorption in strongly bound or chemisorbed states, or as active oxygen compounds (for example, peroxide, superoxide, hydroperoxide) that decompose in a humid atmosphere.

Second, the results of the simulation of the GEX humid mode with RF glow discharge treated soil supports the possibility that the oxygen release seen in the GEX arose from labile oxygen surface species formed during long exposure of the martian soil to ultraviolet radiation induced active species in the atmosphere.

We thank Edward L. Merek, Sherwood Chang, Donald L. De Vincenzi, Vance I. Oyama and Richard D. Johnson of the NASA Ames Research Center, for aid in sample preparation and simulation experiments and helpful discussions.

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Received 19 September; accepted 30 November 1977.

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Energy basis of dielectric relaxation loss

JONSCHER has reviewed the phenomenology of dielectric, mechanical and some magnetic relaxations in a very wide range of materials¹. He found² that a fundamental insight into the physics of such processes may be obtained by considering that the ratio of the energy dissipated to the energy stored in these materials per radian of sinusoidal excitation is a constant which does not change over a very wide range of frequency.

This observation, the so-called 'universal dielectric response', has been derived by examining the behaviour of dielectric loss in the frequency and time domains, and tentatively modelled in terms of a partial screening mechanism²⁻⁴. Here we shall discuss Jonscher's work within the context of earlier work, and it will be shown that the presence of a loss peak in the frequency domain, or a change in the slope of the double logarithmic time-domain graph, need not require the two consecutive processes in the material that have been thought to be necessary^{1,4}. Subsequently, the clarified 'universal dielectric response' is used to interpret various empirical loci on the complex permittivity plane, Cole-Cole⁵, Davidson-Cole⁶ and Williams-Watts⁷⁻⁹ plots, and to compare these loci with the simple Debye form in terms of the behaviour of a single 'storage parameter', s , replacing Jonscher's n and m and the previously physically uninterpreted α of Cole and Cole and β of Davidson and Cole.

The classical Debye theory of dielectric relaxation¹⁰ involves the orientation of polar molecules in an applied field being resisted by the viscous damping of this rotation, as the molecules are considered as spheres in a continuous medium having the macroscopic viscosity of the material. On the complex relative permittivity plane

$$D^*(\omega)/\epsilon_0 E = \epsilon^*(\omega) = \epsilon'(\omega) - i\epsilon''(\omega) = 1 + \chi^*(\omega) \quad (1)$$

where ϵ_0 is the permittivity of free space, $8.845 \times 10^{-12} \text{ F m}^{-1}$, and $\chi^*(\omega) = \chi'(\omega) - i\chi''(\omega)$ is the susceptibility, the locus of ω predicted by the Debye theory is semicircular, with

$$\epsilon^*(\omega) - \epsilon_\infty = (\epsilon_s - \epsilon_\infty)/(1 + i\omega\tau) \\ = (\epsilon_s - \epsilon_\infty)/(1 + \omega^2\tau^2) - i(\epsilon_s - \epsilon_\infty)\omega\tau/(1 + \omega^2\tau^2) \quad (2)$$

In 1941 Cole and Cole⁵ noted that the dispersive mechanisms then used to explain dielectric relaxation loss, namely this Debye viscous damping or alternatively direct current conductivity, had the common characteristic of being wholly dissipative in nature, and that this was represented in the equivalent circuit of the dielectric by a pure resistance (Fig. 1a). However, they also showed that the behaviour of a very large number of dielectrics produced not a semicircle on $\epsilon^*(\omega)$, but a minor arc of a circle having its centre 'below' the real axis, described by

$$\epsilon^*(\omega) - \epsilon_\infty = \frac{(\epsilon_s - \epsilon_\infty)}{1 + (i\omega\tau)^{1-\alpha}} \quad (3)$$

and requiring that the dispersive mechanism producing the loss should not be represented by the purely dissipative Debye resistor, but rather by a complex 'polarisation impedance' $R(i\omega\tau)^{-s}$, Fig. 1b. The loss peak produced in consequence by the Cole-Cole equivalent circuit is described by

$$\epsilon''(\omega) = \chi''(\omega) = \frac{(\epsilon_s - \epsilon_\infty) [\cos(\alpha\pi/2) (\omega\tau)^{1-\alpha}]}{1 + 2 \sin(\alpha\pi/2) (\omega\tau)^{1-\alpha} + (\omega\tau)^{2(1-\alpha)}} \quad (4)$$

which can be compared with the Debye term in equation (2). Both loss peaks are illustrated in Fig. 2.

In the words of Cole and Cole⁵, using the polarisation impedance "implies a conservation or 'storage' of energy in addition to dissipation of energy in the mechanism of molecular interaction responsible for the dispersion". As the vacuum response of the material is accounted for within the small parallel capacitive branch of the equivalent circuit, when

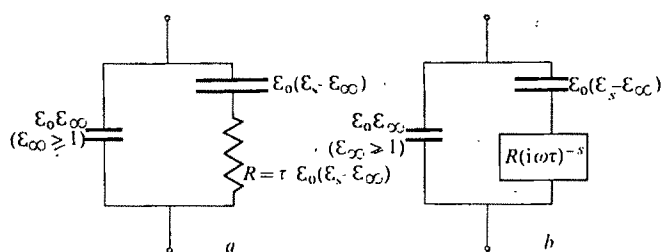


Fig. 1 Equivalent circuits of dielectric relaxation processes: a, Debye; b, Cole-Cole: s is a constant, α ; general: s may be a function of $(\omega\tau)$.

considering the properties of the boxed element in isolation we may write that the dispersive mechanism has

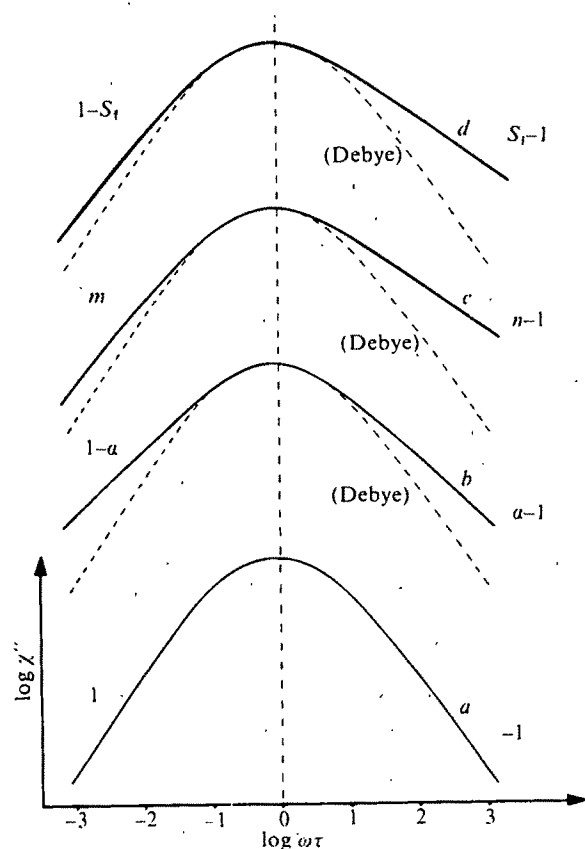
$$\epsilon_0 \epsilon'_b = \epsilon_0 \chi'_b = \epsilon_0 (\epsilon_s - \epsilon_\infty) \sin(\pi/2) (\omega\tau)^{s-1} \quad (5)$$

$$\epsilon_0 \epsilon''_b = \epsilon_0 \chi''_b = \epsilon_0 (\epsilon_s - \epsilon_\infty) \cos(\pi/2) (\omega\tau)^{s-1}$$

$$\text{and hence } \epsilon''_b/\epsilon'_b = \chi''_b/\chi'_b = \cotan(\pi/2) \quad (6)$$

where s is a storage parameter equal in the Cole-Cole dielectric to a constant value, α . Consequently Cole and Cole⁵ concluded that "the ratio of the average energy stored to the average energy dissipated per cycle in the form of heat is a constant independent of frequency". This principle leads to a constant phase-angle, $(-\pi/2)$ in the case of the polarisation impedance, as was first realised according to Cole¹¹ by Irving Wolff in the late 1920s. It also follows^{10,12} from the pioneering nineteenth century time-domain investigations of dielectric loss by Hopkinson, who produced evidence of power-law decay corresponding to parts of Fig. 3, as did Kohlrausch less completely in 1854.

Fig. 2 Frequency domain: the symbols show the slopes of the lines to which the loss peaks are asymptotic. The peaks are vertically separated for clarity. a, Debye; b, Cole-Cole; c, Jonscher; d, general.



Jonscher has discussed the principle in terms of Hilbert transforms¹³, calling it the 'universal dielectric response'¹. He has made^{2,3} the useful extension

$$\varepsilon''_b/\varepsilon'_b = \chi''_b/\chi'_b = (1-p)/p = \cotan(s\pi/2) \quad (7)$$

where p is the proportion of energy stored during a cycle of the field. However, the storage of a constant proportion of energy during each cycle of the field probably results from the storing of a constant proportion of energy during each of the operations of the dispersive mechanism that can take place before the field-reversal at the end of a half-cycle of the applied frequency. It is, therefore, more fundamental to consider each operation of the dispersive mechanism itself. If during this operation a proportion p of the energy is stored (Fig. 4), then equation (7) follows. Of course, if all the energy is dissipated, then the Cole-Cole α is zero, as the polarisation impedance reverts to the Debye resistor when p and s go to zero (cf. ref. 3). The Debye case (Fig. 2a) is the limit of zero energy storage within

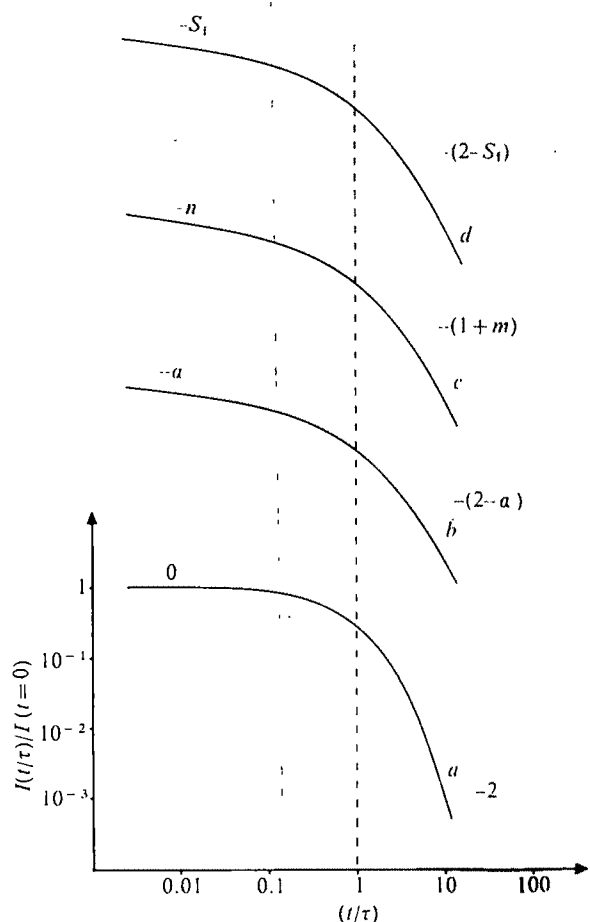


Fig. 3 Time domain: the symbols again show the slopes of the lines to which the curves are asymptotic. The non-Debye curves are vertically displaced for clarity. a, Debye; b, Cole-Cole; c, Jonscher; d, general.

the dispersive mechanism. However, unlike the situation in the viscous rotation of the Debye dipole sphere, in most real systems the necessary work will probably be used to separate the moving charge (for example, a pinned dipole^{2,3}) from its counter-charge atmosphere of partial screening, as described by polaron theory^{14,15}, and there will therefore also be some stored energy involved in the system due to the polaronic nature of the moving charge carrier. Although a theoretical investigation of the time course of the local amount of such stored energy might be somewhat difficult, the constant ratio of equation (7) follows

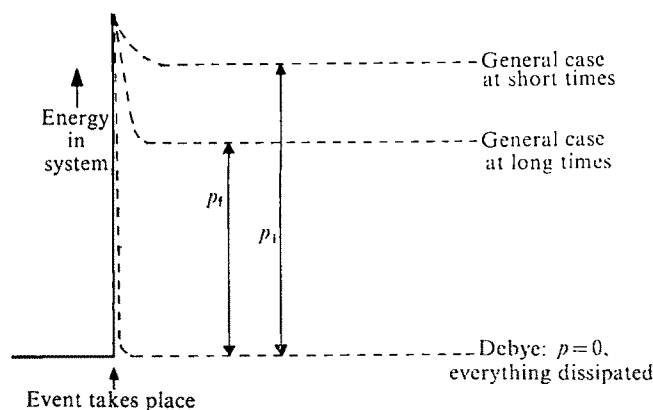


Fig. 4 Schematic diagram of the inferred progress with time of energy storage within an operation of the dispersive mechanism.

when there is inevitably involved, during each dipole jump and operation of the dispersive mechanism, a certain amount of energy storage associated with a certain amount of energy dissipation, and the field has, of course, to supply both. A change in the frequency of the field may affect the numbers jumping, but not the proportionality between the requisite dissipation and storage of energy related to these jumps. If no energy is stored and all is dissipated, we revert to the Debye case, Fig. 2a.

A single and unchanging proportion of storage p corresponding to a single value of s ($= \alpha$) can therefore lead to a definite (and broadened) loss peak, Fig. 2b, without involving two different and consecutive relaxation processes^{1,4}, which thus removes the only apparent enigma hindering the progress of a Jonscher-type interpretation of relaxation loss. The associated change in the exponent of the power-law loss with frequency, equation (4) and Fig. 2b, is both empirically necessary and theoretically acceptable: the Cole-Cole equation (3) has been shown⁵ to obey the Kramers-Kronig relations, modelling the entire necessary equivalent circuit (Fig. 1b) rather than just the polarisation impedance of the particular Hilbert transform demonstration of Jonscher¹³.

In the time domain Cole and Cole¹² further showed that to have not only a finite initial charge as elapsed time t tends to zero, but also a finite total charge transfer as t tends to infinity, the power-law decay of current resulting from step-potential stimulation has to have a knee (Fig. 3). As this occurs for a single value of energy storage p corresponding to a single value of s ($= \alpha$) across the time range, and applies also in the Debye case of $p = s = 0$, again there is no need to postulate two consecutive processes⁴ as, of course, is required to agree with the frequency domain simplification, the loss peak in the frequency domain corresponding to the knee in the time domain.

The asymptotic slopes of the knee, however, are not always those to be expected from the Cole-Cole theory¹ (unless Jonscher's $(n-1)$ equals his m)³. This discrepancy is confirmed by much experimental data^{3,7-9}, and is extremely important as the inference is that p cannot be assumed to be constant in all cases across an arbitrarily wide range of time compared with the time constant τ of the relaxation mechanism. The initial proportion p_i (Fig. 4) and the 'final' proportion p_f correspond through equation (7) with values s_i and s_f of the storage parameter s that are manifested in the time domain as the general slopes of Fig. 3, and in the frequency domain as the asymmetrically broadened loss peak Fig. 2d. This concept will be elaborated quantitatively elsewhere¹⁶, and the required form of decay of p shown to be reasonable.

Meanwhile, let us briefly and qualitatively compare events in the time domain with their results on the permittivity plane, taking t as the reciprocal of the applied frequency $\omega/2\pi$ and assuming the absence of d.c. conductivity, which would mask the dielectric relaxation behaviour. Then if p decays to zero

extremely quickly, before even the time corresponding to t/τ very small ($\omega\tau$ very large), we find Debye results, a semicircle, on $\epsilon^*(\omega)$. If at least p decays to zero by the time corresponding to t/τ very large ($\omega\tau$ very small), then we find Davidson–Cole⁶ results on $\epsilon^*(\omega)$; the Davidson–Cole parameter β will be interpreted elsewhere¹⁶. But if p has not fully decayed to zero by this time corresponding to t/τ very large ($\omega\tau$ very small) we see something like Williams–Watts^{7–9} behaviour on $\epsilon^*(\omega)$, and if p does not decay at all from its initial value by the time that t/τ is very large, then Cole–Cole behaviour is seen on $\epsilon^*(\omega)$. Such effects can either be deduced intuitively by comparing Fig. 2 of ref. 12, with those given here, or derived analytically¹⁶. Thus combining the work of Cole and Cole with Jonscher's effective demonstration of the inconstancy of their parameter α , qualitatively explains all the experimentally observed types of dielectric relaxation. Quantitative results can be expected to advance with polaron theory.

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Received 26 August; accepted 21 November 1977.

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Pressure retardation of vitrinite diagenesis, offshore north-west Europe

PHYSICO-CHEMICAL processes that increase coal rank also increase vitrinite reflectance, or the level of vitrinite diagenesis. The principal physical factors controlling these processes have been identified as geothermal gradient, thermal conductivity of lithological section, duration of heating, and pressure^{1,2}. Interaction of these factors on coalification has not been demonstrated conclusively in nature or by laboratory experiments, but temperature and time have been generally regarded³ as most influential. Although the retarding effect of pressure has been widely recognised, the role of pressure is not clearly understood and has been considered³ negligible. Here the same values of mean vitrinite reflectance in oil ($R_{mo}\%$) have been related to present temperature, (T), and to a function of the present temperature and the ratio of the gradients of normal hydrostatic pressure (P_{NH}) and observed pore pressure (P_p), $T(\gamma P_{NH}/\gamma P_p)$, in Fig. 1a and b respectively. Comparison of correlation coefficients of Fig. 1a and b demonstrates that, for values of R_{mo} less than 1.50%, retardation of vitrinite diagenesis by abnormally high formation pressure, is both significant and predictable. As a corollary, the time factor seems to be of limited significance on a geological scale.

For Fig. 1a and b, values of vitrinite reflectance have been restricted to those from coals and non-calcareous shales from several wells offshore from north-west Europe. Sections sampled in these wells do not show evidence of uplift. The samples range in age from mid-Tertiary to Carboniferous. The wide range of ages might not be considered ideal. It is more important, however that present depths of burial and present temperatures are likely to be the maximum suffered by the samples.

The three types of pressure distinguished in geological sections, pore pressure (P_p), effective overburden pressure

(P_{EO}), and geostatic pressure (P_G), are related according to the simple formula

$$P_G = P_p + P_{EO} \quad (1)$$

Following Gretener⁴, geostatic pressure gradient (γP_G) is taken to be 1.00 psi ft⁻¹ (= 0.231 kg cm⁻² m⁻¹). In offshore north-west Europe, the pore pressure gradient of normally pressured sequences, normal hydrostatic pressure, (γP_{NH}) is generally about 0.45 psi ft⁻¹, and gradient of effective overburden pressure (γP_{EO}) is, therefore, 0.55 psi ft⁻¹. Some samples for vitrinite reflectance have come from normally pressured sequences but others are from abnormal, overpressured intervals. In these intervals, γP_p exceeds γP_{NH} , and is generally greater than 0.50 psi ft⁻¹. Conversely, γP_{EO} in the overpressured intervals is less than 0.50 psi ft⁻¹.

Many techniques for estimating formation pressures are available^{5,6}. Formation tests yield the best pressure information, but not enough have been conducted in the wells studied for composition of a satisfactory pressure profile. Therefore, profiles of sonic transit times in shales (Δt_s) penetrated in the wells have been favoured in this study for estimation of formation pressures^{6–8}. Estimates of pressure by means of this technique, provide sufficient values of pressure for plotting pressure profiles of high precision in individual wells.

Values of present temperature (T), in °F, were estimated by interpolation between control temperatures. Horner-type plots of log recorded, bottom hole temperatures⁹ were used to determine control temperatures. During normal compaction of porous, water saturated shales, geostatic pressure increases as depth of burial increases. Decrease in shale porosity and consequent reduction in pore water content relate to increase in geostatic pressure and effective overburden pressure^{10,11}.

Figure 2, based on data from Lewis and Rose¹¹, shows that the coefficient of thermal conductivity of water saturated, porous shale increases with decrease in water content according to the formula:

$$K_t = (K_s K_r \phi / K_s) \quad (2)$$

where K_t is thermal conductivity of total shale; K_s is thermal conductivity of solid shale (= 1.138 Btu h⁻¹ ft⁻¹ °F⁻¹), K_r is thermal conductivity of fluid (water = 0.363 Btu h⁻¹ ft⁻¹ °F⁻¹), and ϕ is shale porosity (where 1 Btu h⁻¹ ft⁻¹ °F⁻¹ = 1.488 kg cal h⁻¹ m⁻¹ °C⁻¹). It is important that thermal conductivity of water does not change significantly with variations in salinity⁶.

In overpressured section, shale porosity (ϕ), effective overburden pressure, and pore-water content are the same as at shallower depths in normally compacted shale sequences. Thus thermal conductivity will be abnormally low in overpressured sequences, whatever the depth, pressures, and porosity of the overpressured shale-water system.

Reduction in thermal conductivity of overpressured shales causes increase in thermal gradient of the overpressured sequence, according to the equation for heat flow¹¹

$$Q = (k_t/L) a \Delta T \quad (3)$$

where k_t is thermal conductivity of shale section, 'L' is thickness of shale section, and ΔT is temperature change along section of thickness 'L', that is the thermal gradient.

Overpressured shale is a heat insulator and normally pressured shale is a heat conductor¹¹. In a state of thermal equilibrium, however, heat flow in an overpressured sequence (Q_{op}) equals that in normally pressured sequence (Q_{np}) of the same section. For this condition the equation for thermal gradient of the overpressured section (ΔT_{op}) is

$$\Delta T_{op} = (\Delta T_{np} (k_{np}/k_{op})) \quad (4)$$

Therefore, the thermal gradient in the overpressured section will be greater than that of the normally pressured section.

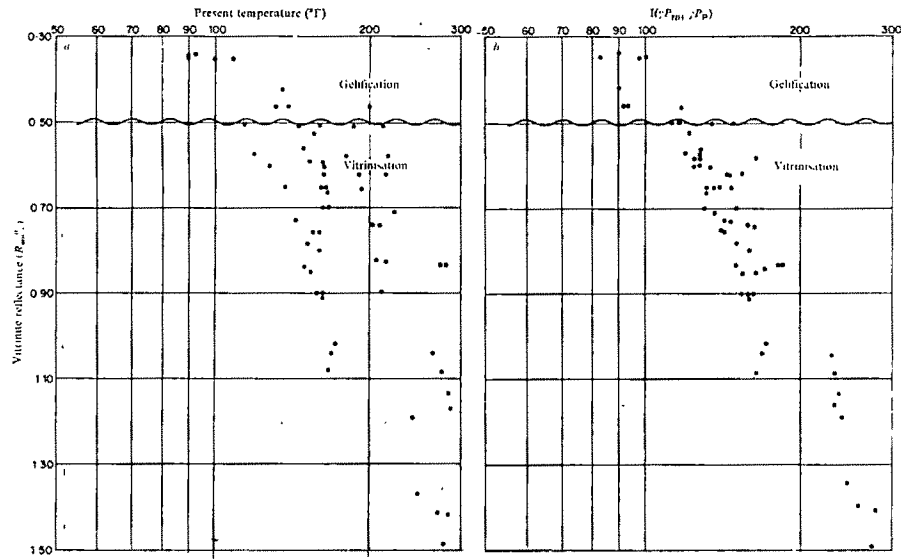


Fig. 1 a, Relationship of vitrinite reflectance ($R_{mo}\%$) to present temperature (T). Temperature in $^{\circ}\text{F}$. Correlation coefficient (r) is 0.716 for the equation $\log_n T = 1.998 + 0.326 R_{mo}\%$. b, Relationship of vitrinite reflectance ($R_{mo}\%$) to the pressure-temperature function ($T(\gamma P_{NH}/\gamma P_P)$). Correlation coefficient (r) is 0.952 for the equation $\log_n (T(\gamma P_{NH}/\gamma P_P)) = 1.838 + 0.433 R_{mo}\%$.

In a subsurface section, the temperature (T_d) at a given depth (D) is determined from the equation

$$T_d = T_s + D \Delta T \quad (5)$$

where T_s is the mean surface temperature. Hence, the temperature at a given depth in an overpressured interval will be greater than the temperature at the same depth in a normally pressured interval for the same type of lithology. Conversely, for a given temperature, heat flow will be lower in an overpressured interval than a normally pressured interval.

The low correlation coefficient ($r = 0.716$) of the relationship of $R_{mo}\%$ to present temperature (T) of Fig. 1a contrasts with the high order of correlation ($r = 0.952$) of the relationship $R_{mo}\%$ to $T(\gamma P_{NH}/\gamma P_P)$ of Fig. 1b. The improved correlation coefficient for the function $T(\gamma P_{NH}/\gamma P_P)$ demonstrates that the influence of abnormal formation pressure must be considered when evaluating the relationship of vitrinite reflectance to natural, subsurface physical conditions.

Coalification has been regarded as a first-order reaction³; the same is probably true of vitrification. In this case, improvement in correlation noted above can be explained readily by reference to the Arrhenius equation

$$k = A \exp(-E/RT) \quad (6)$$

where k is the reaction rate; A is the Arrhenius factor; and $\exp(-E/RT)$ is the Boltzmann factor (= fraction of molecules with energy equal to or greater than the critical activation energy E). From this equation

$$\log_n k = -E/RT + \log_n A \quad (7)$$

where $\log_n A$ is a constant.

By substituting in equation (7) for temperatures T_n (normally pressured) and T_o (overpressured), reaction rates k_n and k_o , at temperatures T_n and T_o , respectively, can be compared according to the formula

$$\log_n (k_o/k_n) = \frac{E}{R} \left(\frac{1}{T_n} - \frac{1}{T_o} \right) \quad (8)$$

where temperatures are expressed in K.

For coeval and identical values of $R_{mo}\%$, one at normal pressure, the other in an overpressured sequence, the tempera-

ture will be higher in the overpressured sequence and (k_o/k_n) will equal 1. However, values of T_n ($\gamma P_{NH}/\gamma P_P$) and T_o ($\gamma P_{NH}/\gamma P_P$) will be the same where T_n and T_o are expressed in $^{\circ}\text{F}$ or in K. This is because ($\gamma P_{NH}/\gamma P_P$)=1 in a normally pressured sequence and ($\gamma P_{NH}/\gamma P_P$)<1 in an overpressured sequence. Therefore from equation 8:

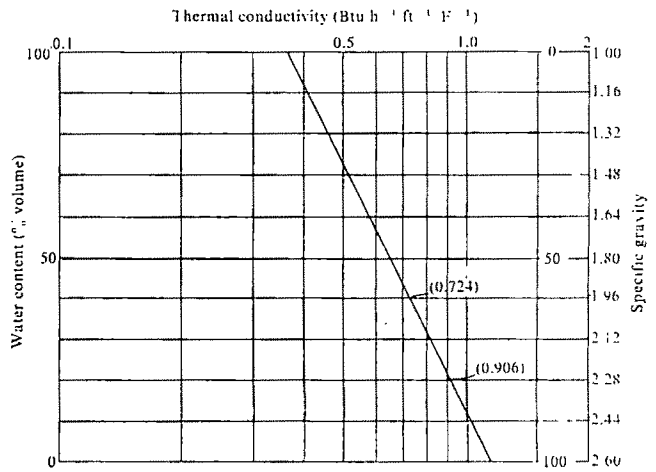
$$\log_n (1.0) = \frac{E}{R} \left(\frac{1}{T_n} \cdot \frac{\gamma P_{Pn}}{\gamma P_{NH}} - \frac{1}{T_n} \cdot \frac{\gamma P_{Pn}}{\gamma P_{NH}} \right) = 0$$

here $T(\gamma P_{NH}/\gamma P_P)$ should be expressed in K.

In Fig. 1b the scatter of points in the gelification stage might indicate a change in the gradient of increase of $R_{mo}\%$. But there are too few points to establish that the rate of increase in $R_{mo}\%$ in the gelification stage is different to that of the vitrification stage, even though physico-chemical processes are different between these stages. In the gelification stage the role of pressure is probably greater than it is in vitrification.

Detailed investigation of the relationship of effective overburden pressure to vitrinite reflectance is still in progress, but

Fig. 2 Relationship of shale water content to thermal conductivity of shale. Increase in thermal conductivity of shale is exponentially related to decrease in water content. Thermal conductivities of 0.724 and 0.905 $\text{Btu h}^{-1} \text{ft}^{-1} \text{ } ^{\circ}\text{F}^{-1}$ relate respectively to shale water contents of 40% and 20%.



preliminary results indicate a closer correlation to $R_{mo}\%$ than that of $T(\gamma P_{NH}/\gamma P_P)$. This may be due to more accurate measure of heating as P_{EO} measures confining pressure, reproducible in closed system laboratory studies. Effective overburden pressure can be determined at the levels of the sample taken for measurement of vitrinite reflectance.

The high coefficient of correlation of Fig. 1b has demonstrated that the combined effect of heat and pressure, especially overpressure, influences increase in vitrinite reflectance for values of R_{mo} less than 1.50%. Pressure has a determinative role in this combination, because of its influence on thermal conductivity of the geological section containing the vitrinite.

Figure 2 and equation (3) indicate that the thermal conductivity of shale in a thick continuous shale section will be the same for a given effective overburden pressure irrespective of its depth. This will be true for any area of uniform heat flow and will be the same whether the shale is overpressured or not.

Heat and time have been related to coalification and increase in vitrinite reflectance. In Fig. 1a and b the values of vitrinite reflectance are for the same samples, so the time-factor does not have to be considered in this comparison. Nevertheless, the comparison has important implications for the significance of time in coalification processes. The amount of time available for heating may influence the final state of maturation of vitrinite, coal or other hydrocarbons. From the excellent correlation of Fig. 1b it can be inferred that the time-factor in relation to precision of vitrinite reflectance estimates for given temperatures and pressures, is of limited significance on a geological scale and is likely to be critical only for a short period.

Further investigation of the combined effect of heat, pressure, thermal conductivity of sediment, and time in natural, geological conditions will improve understanding of the increase in reflectance of vitrinite. The resulting data should also provide a more sound basis for interpretation of the role of natural physical processes in the maturation of all types of organic matter in terms of physical chemistry.

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Received 16 May; accepted 25 November 1977.

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Rejecting evidence of Gothenberg geomagnetic reversal in New Zealand

MORNER and Lanser¹, in 1974, gave evidence that the Gothenberg Geomagnetic Event had been discovered in New Zealand. The report was made prematurely, being based on remarks by Topping² in an address at the INQUA Congress in Christchurch, New Zealand, 1973. Further investigations, detailed here, show that the presumed evidence for the Gothenberg reversal was unsound.

Topping's presumption was based on measurements of palaeomagnetic polarities in 16 andesite boulders taken from two adjacent localities in a lahar. The lahar is between 9,540- and 12,450-yr-old (ref. 2) and is located at Bruce Road on the northwestern side of Mount Ruapehu, National Park, New Zealand. Eleven of the boulders displayed

reversed polarities, and Topping concluded that this was sufficient to prove that the lahar was hot when it flowed, and the boulders had not cooled to below their Curie temperatures until after the lahar had come to rest. The independently determined age of the lahar, based primarily on ¹⁴C measurements³, indicated correlation of the implied reversed geomagnetic field with the Gothenberg Event. If correct, this would have been the first record of the Gothenberg Event in the Southern Hemisphere, and in view of its importance more thorough investigation was obviously needed.

Therefore an additional 16 oriented boulders of andesite were taken from one of Topping's localities. Two, three or four samples, at different distances between the outer surface and centre, were taken from each boulder, and polarities were measured with a spinner magnetometer.

Only a few samples had been magnetically cleaned by Topping. They showed no sign of instability after thermal demagnetisation at 150 °C, and Topping assumed that the magnetisations of all his sample boulders were stable. The following more rigorous cleaning tests confirm that Topping's assumption was correct.

Samples from three of the newly collected boulders were thermally demagnetised step by step at 50° intervals between 100 °C and 600 °C, and samples from two others were a.c. demagnetised at peak field intervals of 10 mT up to 50 mT. Directional changes were negligible up to 500 °C for thermal demagnetisation and up to 50 mT for a.c. demagnetisation. The palaeomagnetic stability index of Briden³ was applied and confirmed these observations. From these stability indices a peak field of 10 mT was chosen as best for a.c. cleaning and a maximum temperature of 300 °C for thermal cleaning. The remaining samples were cleaned at either of the above values, approximately half by each method.

The magnetisation directions of samples from each individual boulder are in good agreement with one another. This shows that the material in each boulder was at rest while cooling from the Curie temperature.

The magnetisation directions of individual boulders seem to be random, showing that their natural remanent magnetisation (NRM) could not have been acquired by cooling after coming to rest in the lahar mound.

After cleaning, the mean inclination for all the boulders was –10° with a standard deviation of 35°, and the mean declination was 132° with a standard deviation of 72°. A *t*-test shows that the inclinations and declinations are randomly distributed at the 95% confidence level.

It is thus concluded that the volcanic material composing the boulders acquired its thermoremanent magnetisation (TRM) by cooling in a stable magnetic field before the boulders were involved in the lahar flow. During the lahar flow the rocks were below their Curie temperature, and when they came to rest were magnetically oriented in random directions. They therefore do not record a geomagnetic field direction and are not suitable for testing the existence in the Southern Hemisphere of the Gothenberg Reversed Event.

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Non-biogenic fixed nitrogen in Antarctica and some ecological implications

TOTAL fluxes of nutrients in ecosystems and the biosphere have been estimated¹⁻³, especially for tropical and midlatitude regions, but no study has attempted to estimate the contribution of fixed nitrogen from the Antarctic ice sheet. Using data from analyses of South Pole ice, we estimate here that the mean annual accumulation of fixed nitrogen in the Antarctic ice sheet is 2.73×10^4 tonnes of nitrate (as N) and 1.88×10^4 tonnes of ammonium (as N). If annual snow accumulation approximates annual losses through calving of icebergs, blowing snow and melting of the bottom of ice shelves, then Antarctic Ocean surface waters will be enriched by 4.61×10^4 tonnes of $\text{NO}_3^- + \text{NH}_4^+$ during the austral summer. This contribution of fixed N to circumpolar surface waters may significantly influence productivity.

A total of 364 South Pole ice core samples from various depths were analysed for ammonium ion (phenol-hypochlorite method⁴), nitrate ion (diazotisation method⁵), and nitrate ion (cadmium reduction method⁵). These methods do not detect other reduced NH_x species which might be present in trace amounts. Details of corehandling and analytical methods are reported in Parker *et al.*⁶

Table 1 summarises our results. Almost no NO_3^- was found except in the upper few metres of snow contaminated by fuel combustion products⁶. Average annual accumulation rate estimates at the South Pole range over 6–10 cm of water⁷⁻¹⁰. Based on estimates of annual accumulation rates at various points, an average value of 10 cm of water per year for the entire ice sheet seems reasonable¹⁰. The continent measures $14\text{--}15 \times 10^6$ km² and is > 95% ice-covered with an estimated mean ice thickness of 2.0 km. Using 14×10^6 km² as the ice sheet area, total glacial ice volume is about 28×10^6 km³ with a mean density of nearly 1.0. The assumption that our measured nitrogen values are representative of the entire mass of ice is probably conservative, because coastal Antarctica receives additional nitrogen input from sources such as birds and coastal nitrogen-fixing biota. We calculate the following, assuming a mean density of 1.0 for glacial ice:

- (1) Mean annual NO_3^- input to the continent,
 $(14 \times 10^6 \text{ km}^2) \times (10^6 \text{ m}^2 \text{ km}^{-2}) \times (19.5 \mu\text{g NO}_3^- \text{ l}^{-1}) =$
 $2.73 \times 10^{10} \text{ g} = 27,300 \text{ tonnes} (= 121,000 \text{ tonnes of NO}_3^-).$
- (2) Mean annual NH_4^+ input to the continent,
 $(14 \times 10^6 \text{ km}^2) \times (10^6 \text{ m}^2 \text{ km}^{-2}) \times (13.4 \mu\text{g NH}_4^+ \text{ l}^{-1}) =$
 $18,800 \text{ tonnes} (= 24,100 \text{ tonnes of NH}_4^+).$
- (3) Mean annual $\text{NO}_3^- + \text{NH}_4^+$ input to the continent,
 $(1) + (2) = 46,100 \text{ tonnes} (= 145,100 \text{ tonnes of NO}_3^- + \text{NH}_4^+).$
- (4) Total $\text{NO}_3^- + \text{NH}_4^+$ contained in the Antarctic ice sheet,
 $922 \times 10^6 \text{ tonnes} (= 2.902 \times 10^9 \text{ tonnes of NO}_3^- + \text{NH}_4^+).$

This total fixed nitrogen in the Antarctic ice sheet is nearly five times Delwiche's³ estimate of annual world production from all sources.

Wilson and House¹¹, based on pooled samples of South Pole snow, calculated an annual infall of N (as NO_3^- plus NO_2^-) of 0.0045 lb per acre ($= 7.06 \times 10^6$ kg per year over the continent). Our calculations, which include NH_4^+ , indicate an average of 27.3×10^6 kg or nearly four times their estimate. Wilson and House¹¹ found no NH_4^+ , but they used the direct

Nezzlerisation method, which does not detect NH_4^+ below about $15 \mu\text{g N l}^{-1}$. We agree with Wilson and House that one probable mechanism for nitrogen fixation at the South Pole involves auroral activity, but we further propose that other stratospheric ionisation processes may add to the total fixed nitrogen in polar regions.

Our calculated NO_3^- and NH_4^+ annual input of 46,100 tonnes to the Antarctic ice sheet is only about 0.37% of Delwiche's³ estimate of 7.4 M tonnes per year for the world's atmospheric N fixation. The nitrate- and ammonia-N in Antarctic ice is probably from natural atmospheric sources, a view strengthened by the fact that our analytical data include > 50 m of the South Pole firn core covering over 400 yr (Parker, unpublished). If the primary mechanism of nitrogen fixation is related to stratospheric ionisation over Antarctica, one must expect that a similar condition prevails in the Arctic, although tropospheric mixing patterns will differ in the two regions. Assuming atmospheric N fixation in both polar regions to be approximately equal, then about 0.74% of Delwiche's³ total is produced in polar regions between lat 70° and 90° and > 99% is produced between lat 70°N and 70°S ; this latter zone includes about 80% of the earth's surface and has appreciably less auroral activity. Also, atmospheric nitrogen fixation by lightning^{12,13} and by photochemical oxidation¹³ is considered to be extremely small. Consequently Delwiche's estimate of 7.4 M tonnes per year for world atmospheric NO_3^- production may be excessive. Delwiche³ has emphasised that appreciable uncertainty may result because of difficulties in correctly estimating the proportions, in rainfall, of fixed nitrogen derived from atmospheric ionisation and from land or sea. Our data largely reflect inputs from purely atmospheric processes, because the South Pole is remote from land, sea, and global human activities, also because much of the ice core examined predates the advent of massive industrialisation, and biota such as nitrogen-fixing organisms are absent at the South Pole.

The contribution by the Antarctic ice sheet to the nitrogen balance of the Southern Ocean seems to be significant. The ice sheet is apparently in volumetric equilibrium. Approximately 1.4×10^6 M tonnes per year of N-laden Antarctic ice, therefore, enters Antarctic circumpolar waters either as icebergs or from blowing snow and melting of the bottom of ice shelves. The icebergs contain $19.5 \mu\text{g NO}_3^-$ and $13.4 \mu\text{g NH}_4^+$ l. Using the sum of these mean values, the annual contribution of inorganic nitrogen to the Southern Ocean is 4.61×10^4 tonnes.

Nitrate $-$ N values for Antarctic circumpolar surface waters vary from 0.02 to $21.4 \mu\text{g l}^{-1}$, with a mean value of 2.7 (refs 14, 15). Icebergs should contain about 7.2 times this concentration of nitrate. Little data on NH_4^+ for the Southern Ocean is available. Icebergs are not randomly distributed in Antarctic circumpolar waters, but follow relatively discrete routes dictated by surface currents¹⁶. As icebergs melt, the fresh, low-density water, enriched in nitrogen, will tend to rise and remain at the surface and within the euphotic zone. Nitrogen is recognised to be a nutrient limiting to productivity in some oceanic regions, but is not generally so in Antarctica where higher inorganic nitrogen concentrations occur relative to other seas^{14,15}. We suggest that N-laden ice originating on the Antarctic continent provides a major contribution to the nitrogen budget of the epipelagic zone of the Southern Ocean.

The area of the Antarctic Circumpolar Ocean, between the continental margin and the Subarctic Convergence, is about 14×10^6 km² (ref. 17). The depth of the mixed layer approximates 50 m, so the volume of the Southern Ocean into which the Antarctic nitrogen is injected is about $50 \text{ m} \times 14 \times 10^6 \text{ km}^2 = 7 \times 10^{14} \text{ m}^3 = 7 \times 10^{17} \text{ l}$. Using the estimate of mean NO_3^- concentration ($2.7 \mu\text{g l}^{-1}$) of Balech *et al.*¹⁴, the mixed layer reservoir of NO_3^- amounts to $(2.7 \mu\text{g l}^{-1}) \times (7 \times 10^{17} \text{ l}) = 19 \times 10^{11} \text{ g}$ or 19×10^6 tonnes of inorganic N (neglecting NO_2^- and NH_4^+). We have calculated above that the average annual input of NO_3^- from Antarctica is 2.73×10^4 tonnes, which is 7% of the total in the reservoir.

In spite of the general agreement that phytoplankton pro-

Table 1 Means, standard errors of the mean, and range of values for the two species of fixed nitrogen

Species	\bar{x}	s.e. \bar{x}	Range
NO_3^- -N	19.57	14.34	0-69
NH_4^+ -N	13.34	22.46	0-260

All values are in $\mu\text{g l}^{-1}$

ductivity is light (not nutrient) limited in the Antarctic, this input may have an effect completely out of proportion to its relative size because it is released into the euphotic zone at the time and place most beneficial to phytoplankton (that is, at the surface, as light begins to increase, both promoting photosynthesis and causing the icebergs to melt more quickly).

We thank the USNSF Division of Polar Programs, the core facility at the State University of New York, Buffalo, for the South Pole ice core, Drs R. Cameron, M. A. McWhinnie, and E. Shulenberger for helpful suggestions, and the Office of Naval Research for partial support of this research through grant no. N00014-77-C-0705.

Note added in proof: We have just confirmed that icebergs have concentrations near our estimates and that a concentration gradient occurs, descending with increasing distance from an iceberg.

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Abscisic acid and guard cells of *Commelina communis* L.

THE plant hormone abscisic acid (ABA) is thought to be involved in the regulation of transpiration through its effects on stomatal aperture¹. Application of synthetic ABA causes stomatal closure in whole plants², shoots³ and leaf epidermal strips⁴. During water stress, levels of the hormone increase in plant tissues⁵, including the leaf epidermis⁶, and this is usually associated with stomatal closure⁷. There is evidence that ABA affects the ionic^{8,9} and metabolic⁹ status of the stomatal complex and it has been concluded that ABA acts directly on guard cells in effecting stomatal closure^{8,10,11}. There is, however, little information about the distribution of ABA in specific cells of the epidermis or about the sensitivity of the stomatal complex to ABA. We present here evidence that ABA can accumulate in the stomatal complex of *Commelina communis* L., a species used extensively in studies of stomatal physiology¹². The leaves of this plant yield relatively uncontaminated epidermal strips¹³ and this property has made it possible to calculate the apparent sensitivity of the stomatal complex to ABA. The evidence is based on experiments involving the uptake, distribution and metabolism of exogenous 2-¹⁴C-ABA.

Figure 1 shows that during 1 h when 2-¹⁴C-ABA was supplied through the transpiration stream there was a substantial increase in the radioactivity present in the lower epidermis. Stomatal closure was complete 9 min after application of ABA. These data enable us to calculate an upper limit

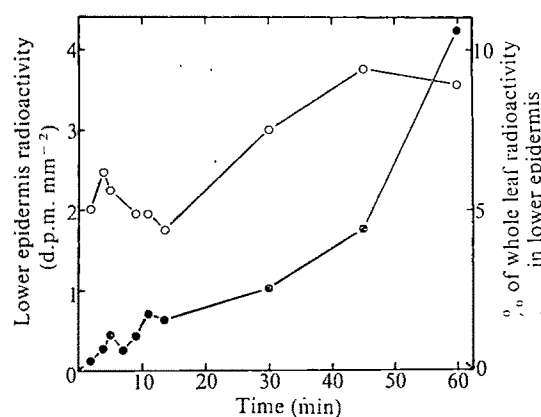


Fig. 1 Uptake of radioactivity by the lower epidermis of *Commelina* leaves fed 2-¹⁴C-ABA through the transpiration stream. Plants of *C. communis* were grown from seed and used when about 7 weeks old. Two-leaved shoots from non-flowering plants were pretreated to obtain open stomata by floating on water through which CO₂-reduced air was bubbled. After drying, they were stood in vials containing 2-¹⁴C-ABA solution (3 μ Ci ml⁻¹, 11.7 Ci mol⁻¹) 250 mm from two 60-W incandescent bulbs at ambient temperatures. Shoots were collected at various times and 50-mm² square abaxial epidermis samples were taken for radioassay (●) and autoradiography. The radioactivity present in the corresponding mesophyll plus adaxial epidermis square was also estimated and used to calculate the percentage of whole leaf radioactivity present in the lower epidermis (○). Radioassay was by liquid scintillation spectrometry after extraction for 3 h in 1 ml methanol:ether (1:1, v:v) mixture. Allowance was made for the quenching caused by the leaf material. Each point on the graph is the mean of three samples from one leaf. Estimates from 20 measurements of stomatal aperture from freeze-dried material indicated that closure was complete after 9 min of 2-¹⁴C-ABA uptake.

for the amount of ABA required to cause stomatal closure. Using an estimate of 50 stomata per mm², we calculate that the stomata had closed after receiving no more than 300 amol of ABA per stomatal complex. Because at the time of closure 5% of the total radioactivity was present in the lower epidermis, this represents 300 fmol per mm² leaf area. This figure confirms the data of Raschke¹¹ who calculated that 90–180 fmol of ABA per mm² leaf area was required to give 5% stomatal closure in *Commelina*. Kriedemann *et al.*¹⁴ estimated that 89–349 fmol of ABA per mm² leaf area was required to initiate closure in French bean, rose and *Zea* leaves.

Microautoradiography (Fig. 2a, b) reveals a distinct correlation between the aggregation of silver grains in the film emulsion and the distribution of stomatal complexes in the tissue. This is not restricted to the pattern of 2-¹⁴C-ABA uptake in isolated epidermes, because similar distributions of silver grains were obtained in autoradiograms from epidermes in which the label was applied through the transpiration stream for 1 h. Evidence from thin-layer chromatography suggests that more than 90% of the radioactivity present in methanol: ether extracts of epidermal tissue incubated in 2-¹⁴C-ABA for 1 h was chromatographically identical to ABA. The mobility of the labelled molecules was manifest in the fact that more than 80% of the radioactivity initially present in such tissue had been lost to the efflux medium after 90 min of incubation in 1 mM CaCl₂ solution. This indicated that distribution studies using ABA should be restricted to the techniques of soluble-compound autoradiography. Neither the net uptake nor the distribution pattern of radioactivity in isolated strips incubated for 1 h was significantly affected by pretreatments causing opening or closing of the stomata.

We have shown that radioactivity from labelled ABA becomes localised in the region of the guard cells of *Commelina communis* after two different treatments. It may be argued that the distribution found is commensurate with that expected of a hormone reaching its target cells. Nevertheless, several points need further clarification, particularly the following. (1) The time courses used for the autoradiography were longer than those required to cause stomatal closure; shorter presentation

times necessitating the use of extremely high specific activity labelled ABA are required. (2) The guard cells seem to be metabolically the most active cells in the epidermis¹⁵ and could be acting as metabolic sinks, although this does not necessarily deny a role for ABA in closure. ABA accumulation in guard cells may represent a form of compartmentalisation or sequestration away from active sites¹⁷. (3) There is evidence that ABA

affects solute distribution between epidermal cells and guard cells¹⁸, but the precise intracellular sites of ABA action remain to be elucidated.

C. I. acknowledges receipt of an SRC senior visiting fellowship.

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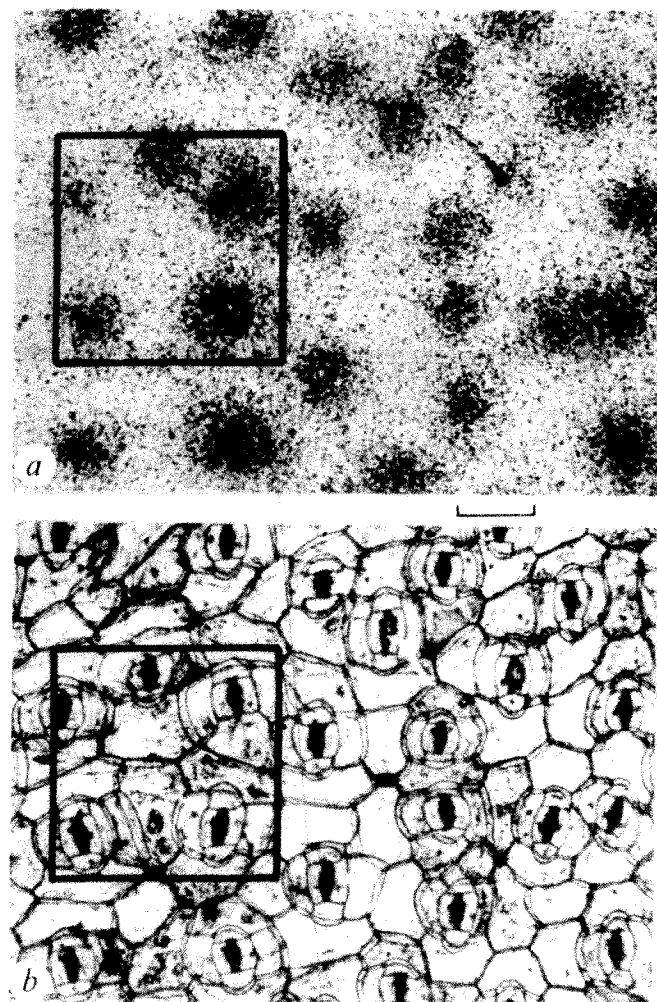


Fig. 2 Distribution of radioactivity in lower epidermis tissue of *C. communis* leaves after floating on $2\text{-}^{14}\text{C}$ -ABA solution for 1 h. **a**, Aggregation of silver grains in the photographic emulsion. **b**, Position of guard cells in the associated freeze-dried *Commelina* epidermis (neutral red stained). $50\text{-}\mu\text{m}^2$ squares of abaxial epidermal tissue were taken from mature leaves of non-flowering shoots. These were floated for 1 h, cuticle uppermost, on 1 mM CaCl_2 solution containing $2\text{-}^{14}\text{C}$ -ABA ($4\text{ }\mu\text{Ci ml}^{-1}$, 11.1 Ci mol^{-1}) maintained at 27°C in a water bath. This solution was gently shaken. After incubation the tissue was rinsed five times for 20 s, with agitation, in 1 ml 1 mM CaCl_2 . Optional prestaining in an aqueous neutral red solution was carried out for 1 min before this. Open stomata were obtained as previously described and closed stomata by floating leaves on water in the dark. Thin-layer chromatography in four solvent systems (benzene:acetone:acetic acid, 70:30:1; ethyl acetate:toluene:acetic acid, 1:10:2; chloroform:methanol, 1:1; *n*-hexane:ethyl acetate, 1:1) was used to estimate radiochemical purity of methanol:ether extracts of epidermal tissue incubated in $2\text{-}^{14}\text{C}$ -ABA solution for 1 h. Efflux of radioactivity was estimated by floating such tissue, cuticle uppermost, on 1 ml of 1 mM CaCl_2 solution for 90 min and estimating the radioactivity present in the epidermis and efflux medium. The autoradiographic procedure was a modification of that used by Willmer *et al.*¹³. Epidermal tissue was applied, cuticle uppermost, to a subbed slide and immersed in liquid nitrogen before freeze-drying for approximately 12 h. The emulsion side of Kodak Pan-F film was applied directly to the tissue and held in place with another slide which was then taped to the first. The film was exposed for 3 weeks in a desiccator at -15°C before development. Controls for pressure artefacts and chemography¹⁶ proved negative scale bar, $100\mu\text{m}$.

Effect of abscisic acid on solute transport in epidermal tissue

AN important aspect of the stomatal mechanism in green plants is the transport of solutes into and out of guard cells in response to stimuli such as illumination¹. The effect of abscisic acid (ABA) in causing stomata to close has been ascribed to ABA-promoted solute leakage from guard cells². The experiments reported here show that leakage alone is insufficient to explain stomatal closure caused by ABA. The involvement of live epidermal cells, acting as recipients of solutes from guard cells, is necessary, as Penny and Bowling have suggested, especially when considering intact leaves³.

To test the requirement for live epidermal cells in ABA-mediated stomatal closure, epidermal strips of *Commelina communis* without living epidermal cells were floated on radioactive ABA in solution. The stomata remained open, but autoradiographs of the strips clearly showed that ABA had accumulated in the stomatal complexes as in untreated strips⁴ (Fig. 1).

We used two approaches to examine the effects of ABA on solute distribution between epidermal and guard cells. Neutral red staining of epidermal strips showed that treatment with ABA resulted in a diminished accumulation of stain in guard cells, and increases in both the rate of accumulation and the final concentration in live epidermal cells. Staining showed that where stomata were adjacent to ruptured epidermal cells the guard cells remained open after application of ABA (Fig. 2). If the transport of neutral red can be taken as an indication of solute transport, one can conclude that in ABA-treated tissue epidermal cells function as active(?) sinks for solutes from guard cells.

For a direct assessment of solute concentrations in epidermal cells, epidermal strips were treated or not treated with ABA while turgor was kept at the optimum epidermal water deficit^{5,6} by floating the tissue on mannitol solution.

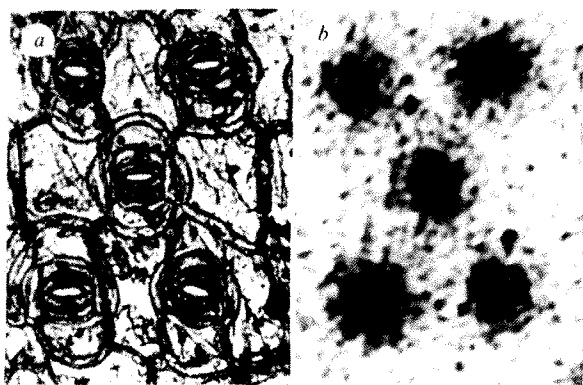
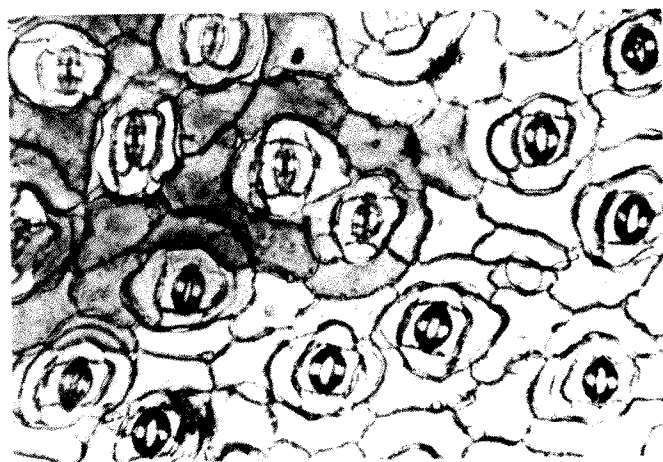


Fig. 1 *a*, Photomicrograph of an epidermal strip of *Commelina communis* with stomata open. The strip had been illuminated while floating on a buffer solution at pH 4.5 to kill the epidermal cells². When stomata had remained open at a steady state aperture for 20 min the strip was transferred for 60 min to a solution of $1 \mu\text{Ci}$ DL-*cis-trans*-2-¹⁴C-ABA (11.7 Ci mol^{-1}). Stomata remained open although ABA had accumulated in stomatal cells as in (*b*). *b*, Autoradiograph of the strips shown in (*a*). Accumulation of ABA in the stomatal cells did not cause stomatal closure.

Under the influence of ABA there was an increase in the concentration of solutes in the epidermal cells probably functioning as recipients of solutes from guard cells. This resulted in a striking difference in the incidence of plasmolysis. In tissue floating on 0.2 M mannitol without ABA almost all epidermal cells were plasmolysed and stomata opened to the maximum extent. In tissue treated with ABA very few epidermal cells were plasmolysed and stomata were closed. This confirmed the indication, gained from neutral red staining, that solutes from guard cells accumulated in adjacent epidermal cells, and of necessity raised their turgor pressure. It could be concluded, however, that the latter played only a secondary role in ABA-mediated stomatal closure because stomata adjacent to slightly plasmolysed epidermal cells nevertheless closed in the tissue treated with ABA.

Although these experiments have clarified part of the mechanism of ABA action on the stomatal complex as far

Fig. 2 Photomicrograph of a microscope field of view ($\times 250$) of an epidermal strip of *Commelina communis*. Stomata had been opened in illuminated leaves kept in CO_2 -free air. ABA was supplied in the transpiration stream for 10 min. Epidermal strips were then taken and stained for 1 min by floating on neutral red (1:20,000), after which they were mounted in liquid paraffin. From the staining it was clear which epidermal cells had been ruptured and which had remained intact. Measurements were made of the effect of ABA on stomatal aperture, the rate of accumulation of dye in the different cells and the final intensity of stain in epidermal and guard cells. Rate of accumulation was measured by photographic recording on colour film. Intensity of stain was measured by transmittance at 600 nm in a Reichert Zetopan using the colour films obtained. Only those stomata which were adjacent to live (stained) epidermal cells had closed.



as solute distribution is concerned, the precise site of that action remains to be demonstrated. The effect could be restricted to the guard cells or may extend to other epidermal cells, especially the subsidiary cells whose exact role in *Commelina communis* is not fully known.

This work was supported in part by a Senior Visiting Fellowship (C.I.) from the S.R.C.

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Characterisation of the surface coat of *Trypanosoma congolense*

THE protozoan parasites *Trypanosoma congolense*, *T. brucei* and *T. vivax* cause extensive disease problems to livestock in Africa¹. In many cases trypanosomiasis results from mixed infections due to all three species². Immunisation has not been successful because of the ability of the parasites to evade the host immune response by changing the antigenic structure of the surface coat³. Chemical and immunochemical characterisation of the surface coat of all three species should give insight into the mechanism of antigenic variation. Recent work on the surface coat of *T. brucei* demonstrates that antigenic variation is the result of a change in structure of a single, variant-specific, surface glycoprotein^{4,5}. No comparable information is available on the structure of the surface coat of *T. congolense* and *T. vivax*. We report here some properties of the surface coat of *T. congolense*.

An isolate⁶ of *T. congolense* (Transmara strain) was supplied by the Kenya Veterinary Laboratories. Clone X4 was derived from the Transmara strain by injecting a single trypanosome into lethally irradiated (900 R) mice. Trypanosomes of this clone were normally grown in irradiated mice and separated from infected blood⁷. Occasionally organisms were collected from normal rats after an additional 3 d passage with no change in surface antigen observable by analytical isoelectrofocusing (IEF), or immunofluorescence using a variant-specific antiserum.

Uncultured or cloned parasites were labelled by lactoperoxidase-catalysed ¹²⁵I iodination or by galactose oxidase oxidation followed by NaBH₄ (³H) reduction. Both enzymes are known to be unable to penetrate the lipid bilayer, and therefore the two methods are commonly used to detect exposed glycoproteins⁸. The adaptation of these labelling procedures to trypanosomes (see legends to Figs 1 and 2) allowed the analyses of surface coat presented here and provided markers for purification of surface material. The labelled coat was extracted by repeated freezing and thawing followed by centrifugation at 230,000g (60 min.). The presence of 1 mM *N*-tosyl-L-lysyl-chloromethane (TLCK) prevented proteolytic breakdown of the labelled material at least during separation into supernatant and pellet fractions at 4°C.

Between 60 and 80% of total radioactivity was present in the soluble supernatant fluid. Most of the remaining radioactivity was solubilised by treating the 230,000g pellet with nonionic detergent NP-40 (Shell) at 0.5% v/v for 30 min at

0 °C and centrifuging at 230,000g (60 min). Total labelled parasites and both aqueous- and detergent-solubilised supernatant fluids were analysed by 7.5–15% gradient polyacrylamide-sodium dodecyl sulphate gel electrophoresis (SDS-PAGE) in reducing and non-reducing conditions¹¹.

Gels were stained with Coomassie blue and destained before detection of radioactive components either by counting 2-mm gel slices or by autoradiography¹². Although a large number of proteins were stained by Coomassie blue throughout the gel length only one major peak containing both ¹²⁵I and ³H was consistently detected by SDS-PAGE of total parasites and soluble or insoluble extracts. The appearance of this peak was unchanged whether the preparations were derived from cloned or uncloned trypanosomes (Figs 1 and 2). The apparent molecular weight of the glycoprotein is 56,000. Gels run in non-reducing conditions gave similar results, and no dimers or oligomers were detectable. A few minor labelled peaks accounting for 1 to 5% of the total radioactivity were also detected by SDS-PAGE and are being analysed. The galactose oxidase catalysed labelling



Fig. 1 Autoradiograph patterns of ¹²⁵I *T. congolense* clone X4 surface proteins. *a*, 10⁷ Parasites solubilised with 2% SDS; *b*, water soluble 230,000g supernatant fluid and *c*, NP-40 soluble 230,000g supernatant fluid from the same number of parasites. The autoradiography shows that the sum of radioactivity in *b* and *c*, does not equal *a*, probably because of loss of material which occurs in separating the major labelled glycoprotein into water soluble and detergent soluble supernatant fluids; in addition a small portion of the radioactivity remains with the detergent insoluble pellet (not shown). The SDS-PAGE slab is 7.5%–15% acrylamide, stabilised with 10% glycerol in the 15% solution. Apparent molecular weights were determined using calibration curves obtained from protein standards subjected to electrophoresis on the same slab gel (see legend to Fig. 2). The method of Marchalonis *et al.*⁸ for enzymatic radioiodination of leukocytes was modified as follows: 2 × 10⁸ washed trypanosomes were resuspended in 50 µl 0.01M phosphate saline buffer, 1% glucose, pH 7.4 (PGS pH 7.4) containing 20 µg of lactoperoxidase (Sigma) and 250 to 500 µCi of Na¹²⁵I (Amersham). The reaction was initiated by the addition of 20 µl of 0.03% H₂O₂ and terminated after 5 min by adding a 100 times excess of PGS pH 8.0 (ref. 7). Two subsequent washes were carried out with the same buffer containing 1% foetal calf serum. Parasite motility was unimpaired at the end of the procedure.

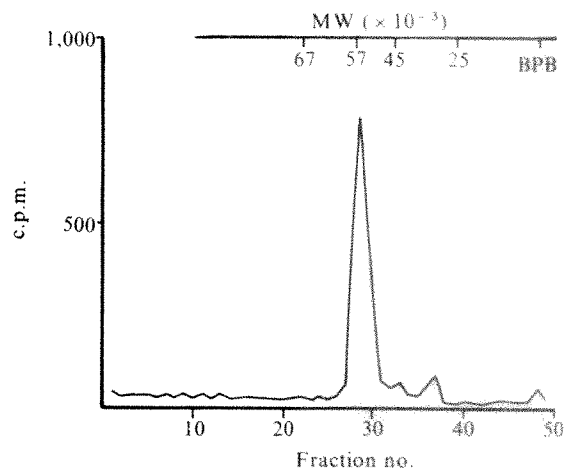


Fig. 2 SDS-PAGE in reducing conditions of 230,000g supernatant fluid from uncloned *T. congolense*. Organisms were labelled on galactose and galactosamine(s) by galactose oxidase oxidation and NaBH₄ (³H) reduction. Molecular weight markers were: albumin (67,000), human IgG heavy chains (57,000), ovalbumin (45,000), human IgG light chains (25,000), bromophenol blue (BPB). ³H labelling of carbohydrates was achieved by modifying the method of Gahmberg⁹. Trypanosomes (2 × 10⁸) in 1 ml PGS pH 7.4 (buffer reference in legend to Fig. 1) were incubated with 1 unit galactose oxidase (Worthington) and 500 units catalase (Worthington) for 30 min. The parasites were then washed with PGS pH 8, resuspended in 1 ml of PGS pH 7.4 and NaBH₄ (³H) 6 Ci mmol⁻¹ (Amersham) was added to give a final concentration of 1 mM and a specific activity of 5mCi per 2 × 10⁸ trypanosomes. The reduction was carried out at room temperature for 15 min with occasional shaking. Termination of the reaction and washes of parasites were as for the iodination method (legend to Fig. 1). Parasites retained normal motility.

technique was used mainly to show the presence of carbohydrate moieties on the surface coat of *T. congolense*. We now routinely use only surface iodination for tracing the parasite coat.

Parallel studies carried out with *T. brucei* (LUMP 227) confirmed previous work showing that the surface coat consists of one major glycoprotein⁴. The apparent molecular weight of the *T. brucei* variant antigen is 62,000. A sample of purified variant antigen from *T. brucei* clone 052 (ref. 4) donated by G. A. M. Cross showed the same molecular weight when measured by the gradient SDS-PAGE technique. In *T. brucei* we also observed the same amount of insoluble glycoprotein as in *T. congolense*. The small difference in apparent molecular weight between the major surface glycoprotein of *T. congolense* and *T. brucei* could have one of several explanations, for example, a peptide bond of the *T. congolense* antigen might be subject to specific proteolytic degradation, or the extent of glycosylation might be very different in the two species.

Supernatant fractions of iodinated cloned and uncloned *T. congolense* were analysed by IEF in polyacrylamide gels. Uncloned material showed many peaks ranging in pI from 5 to 8. It is known that the surface coat glycoprotein of *T. brucei* is variant specific and in fact iodinated supernatant fluids from various *T. brucei* clones always showed one homogeneous peak with a specific isoelectric point (for example Fig. 3b). One might expect similar results for *T. congolense*; however, three peaks were resolved by IEF of a labelled supernatant fraction from clone X4 of *T. congolense* (Fig. 3a). Preparative purification of the surface glycoprotein from 2 × 10¹⁰ cloned parasites⁴ yielded about 1 mg of each of the three components (pI 6.2, 6.4 and 6.6).

An antiserum prepared in rabbit against the IEF peak of pI 6.6 stained > 99% of the parasites using immunofluorescence and showed a single precipitin line in immunodiffusion. Both aqueous- and detergent-soluble supernatant fluids were tested in immunoprecipitation as follows: supernatant fluids derived from 10⁶ parasites were incubated with

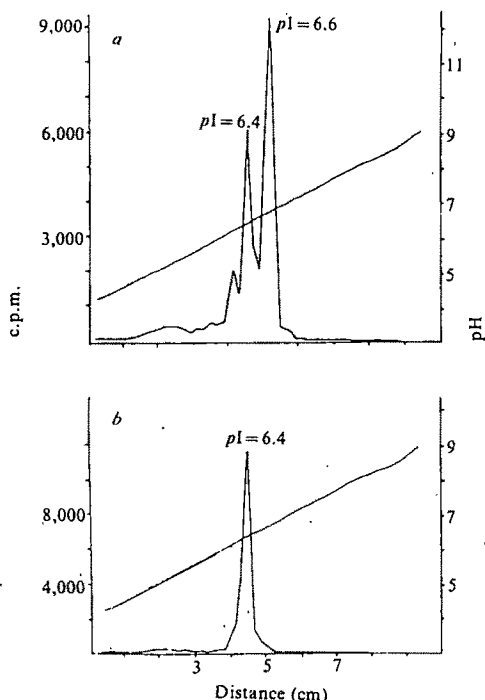


Fig. 3 *a*, A radioiodinated clone of *T. congolense* (X4) was freeze-thawed three times in water and separated into 230,000g (60 min) supernatant and pellet fractions. The supernatant fluid was dialysed against water at 4 °C then run across the width of a standard polyacrylamide gel isoelectrofocusing plate (pH 3.5–9.5) supplied by LKB. The gel was stained for protein with Coomassie blue and destained in ethanol–water–acetic acid (6:13:1). Radioactivity measurements were made on 2 mm fractions. (*b*), As (*a*) except that the radioiodinated trypanosomes were a clone of *T. brucei*.

rabbit anti-variant specific antiserum or normal rabbit serum as control for 30 min at room temperature. An optimal amount of sheep anti-rabbit immunoglobulin serum was added and the mixtures left at 4 °C overnight. Precipitates were washed three times with buffered saline, dissolved in 0.08 M Tris pH 6.8, 0.1 M dithiothreitol, 2% SDS and boiled for 5 min before electrophoresis. SDS-PAGE analysis of the specific precipitates showed the same 56,000 radioactive peak seen in Figs 1 and 2, as well as the protein with pI 6.4 which has a lower molecular weight (data not shown). No radioactive material was precipitated by normal rabbit serum.

These data suggest that an antigenically homogeneous clone of *T. congolense* yields a single specific glycoprotein as surface antigen and that the portion which is detergent-solubilised is immunologically indistinguishable from the soluble glycoprotein, although it possibly has a different attachment to the trypanosome plasma membrane. This might reflect a precursor-product relationship.

The heterogeneity of surface-labelled glycoprotein from cloned *T. congolense* observed from IEF is likely to be due to proteolytic degradation. Breakdown of the 56,000 molecular weight protein was observed even during separation of broken cells into supernatant and pellet fractions at 4 °C in the absence of TLCK. The extent of degradation was enhanced by any further purification procedures, and the final yield of purified variant antigen is far from satisfactory. Improvements of the solubilisation and purification procedures are in progress and will be published elsewhere.

There are several overall similarities between the surface coats of *T. congolense* and *T. brucei*. In both cases one glycoprotein is predominantly accessible to surface labelling; the distribution of surface glycoprotein between water soluble and insoluble fractions of broken cells is similar, as are the molecular weights. These results suggest the need

for comparable studies on *T. vivax* to provide a more unified understanding of basic surface coat structure in African trypanosomes.

We thank Ms J. Vernooij and Mr G. Mpimbaza for technical assistance.

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Behavioural mutants of *Paramecium caudatum* with defective membrane electrogenesis

THE swimming behaviour of *Paramecium* is mostly dependent on the activity of its cilia¹, which are regulated by the intracellular cation concentration, which in turn is modified by Ca-dependent electrogenesis in the membrane^{2,3}. Behavioural abnormalities due to defective membrane electrogenesis have been found in mutants of *P. tetraurelia*^{4–7}. We have now found similar abnormalities among a group of behavioural mutants of *P. caudatum*. These show promise of an excellent source of genetic markers.

Unlike *P. tetraurelia*, *P. caudatum* does not exhibit natural autogamy⁸, and is therefore a less convenient source of homozygous clones. Strain Kyk201 (mating type VI of syngen 3), however, which we used, shows frequent selfing conjugation so that recessive mutant genes should be expressed. The progeny of selfing conjugations show a high rate of survival.

P. caudatum in the logarithmic phase in fresh lettuce medium with *Klebsiella aerogenes*⁹ were treated with the mutagen *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine (10 µg ml⁻¹) for 40 min and then cloned. After the isolated cells had divided 12–15 times, clones were starved to induce selfing conjugation¹⁰. Those containing many conjugating pairs were fed again with bacterised medium to induce five to eight more cell divisions. Then the progeny were subjected to galvanotactic and geotactic¹¹ screening. Every cell which showed abnormal swimming behaviour in an electric field was pipetted into a separate dish to make an independent clone. The subsequent progeny were compared with the wild type with respect to swimming behaviour in response to various stimuli and with respect to electrophysiological characteristics.

The wild type makes an avoiding response due to a transient ciliary reversal when it encounters an obstacle. It may also do this spontaneously. It makes repetitive avoiding responses at fairly regular intervals in a mixture of Ba²⁺ and Ca²⁺ (ref. 12) ('barium dance', Fig. 1*a*), and undergoes longer term ciliary reversal (about 1 min) when K⁺ is above a certain concentration in the external medium¹³. Depolarisation of the membrane by an outward electric current causes ciliary reversal in the wild type¹⁴. This reversal is always associated with an inflow of Ca²⁺ through the membrane due to an increase in Ca permeability^{2,3}. Ca is a mediator for the coupling of membrane permeability with ciliary reversal¹⁵.

As summarised in Table 1, none of these behavioural responses due to ciliary reversal was seen in mutant 13B

Table 1 Swimming behaviour and ciliary responses to various stimuli in mutants of *P. caudatum*

Strain	Swimming velocity (µms ⁻¹)	Avoiding response	Escape response	Ciliary reversal by outward current	Ciliary augmentation by inward current	Ciliary reversal by K ⁺ (s)	Barium* dance
Wild type							
Kyk201	1384 ± 27	+	+	+	+	43.6 ± 4.8	+
Mutants							
13B (CNR)	1548 ± 27	—	+	—	+	0	—
13D (K ⁺ sensitive)	1108 ± 32	+	+	+	+	255.6 ± 31.0	—
11C (slow swimmer)	864 ± 16	+	+	+	+	26.3 ± 6.1	—

+ and —, Presence and absence of the response respectively. All measurements were made at room temperature (20°–25 °C). Swimming velocity was measured by Dryl's method¹² in a mixture of 4 mM K⁺, 1 mM Ca²⁺ and 1 mM Tris-HCl (pH 7.2). The duration of ciliary reversal was measured when the specimen was transferred from a mixture of 1 mM K⁺, 1 mM Ca²⁺ and 1 mM Tris-HCl (pH 7.2) to a mixture of 20 mM K⁺, 1 mM Ca²⁺ and 1 mM Tris-HCl (pH 7.2). The figures represent the average and standard error of 10–20 measurements in different specimens.

* Repetitive avoiding response in association with all-or-none type action potentials shown in a mixture of Ba²⁺ and Ca²⁺.

(Fig. 1*b*). It showed no ciliary reversal in response to various stimuli. Electrophysiological examination revealed that, in contrast to the wild type^{16,17}, it produced no regenerative Ca action potential in response to a depolarising current (Fig. 2*a* and *c*). Only a residual active Ca²⁺ response was detectable when the current was very strong and the potential response was displayed in a form of phase plane trajectory¹⁸ (Fig. 3*a*, *b*, *d* and *e*). However, ATP-reactivated cilia of Triton-extracted specimens of 13B (which have a disrupted cell membrane) were reversed in their beating orientation when the Ca²⁺ concentration in the reactivation medium was raised above 5 × 10⁻⁶M, as happens with similarly treated wild type specimens^{15,19}. Externally applied Ca has direct access into such cells and can influence the Ca²⁺-sensitive reversal mechanism of cilia without restriction by the membrane. Thus, the reversal mechanism of the cilia is functional in mutant 13B, and the behavioural deficiency is probably due to a defect in the depolarisation-sensitive Ca gating system in the membrane. We shall now refer to mutant 13B as CNR (caudatum non-reversal).

A transient increase in forward swimming speed due to increased beat frequency of the cilia in the normal direction (ciliary augmentation) was observed in CNR, as in the wild type, in association with a hyperpolarising mechanoreceptor potential after mechanical stimulation of the posterior membrane²⁰ (Table 1, Fig. 2*f* and *h*). An injection of inward current into a CNR cell always caused ciliary augmentation in

association with an increase in K⁺ permeability of the membrane, as in the wild type²¹ (Table 1, Fig. 2*b* and *d*, Fig. 2*c* and *f*). Thus both the mechanosensitive K gating system in the posterior membrane and the hyperpolarisation-sensitive K gating system in the general membrane remain intact in the CNR specimen.

Mechanical stimulation of the anterior membrane of a CNR cell induced a transient depolarising mechanoreceptor

Fig. 2 Responses of the membrane potential to constant current stimulation (*a–d*) and mechanical stimulation (*e–h*) in *P. caudatum*. *a*, *b*, *e* and *f*, Wild type specimen; *c*, *d*, *g* and *h*, CNR mutant. *E_m*, membrane potential: dashed lines show the resting level, and upward and downward deflections correspond to depolarisation and hyperpolarisation of the membrane, respectively. *S_i*, transmembrane current: dashed lines show zero current level, and upward deflection means outward current while downward deflection means inward current. *S_m*, deflection in the trace shows the duration and relative intensity of the electric pulse activating a piezoelectric crystal to drive a glass rod against the surface of the cell. The horizontal line in the centre of the figure corresponds to 20 ms in *a*, *b*, *c* and *d*, and to 40 ms in *e*, *f*, *g* and *h*. The vertical line corresponds to 20 mV for *E_m*, 4 nA for *S_i* and to 2 V s⁻¹ for *E_m*.

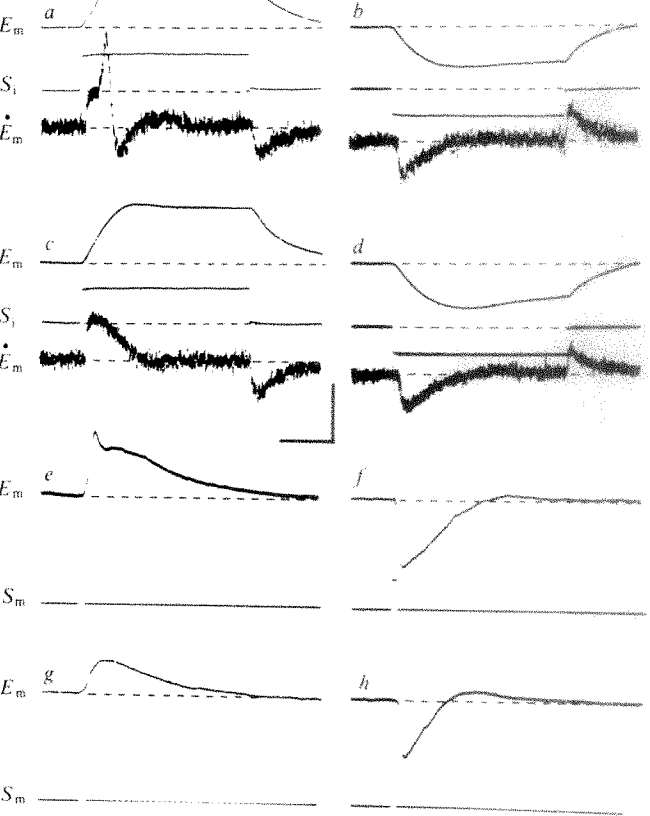


Fig. 1 Swimming behaviour of *P. caudatum* in a mixture of 1 mM BaCl₂ and 1 mM CaCl₂ (buffered to pH 7.2 by 1 mM Tris-HCl). The photographs were taken under dark-field illumination with an exposure time of 4 s (room temperature 25 °C). *a*, Wild type specimens (strain Kyk201) show repetitive avoiding response, which makes the specimen follow a zig-zag or star-shaped swimming path (barium dance). *b*, CNR mutants (strain 13B) do not dance, but continue to swim forward following a long-pitched, spiral path similar to that followed by the unstimulated wild type. The bar corresponds to 1 mm.

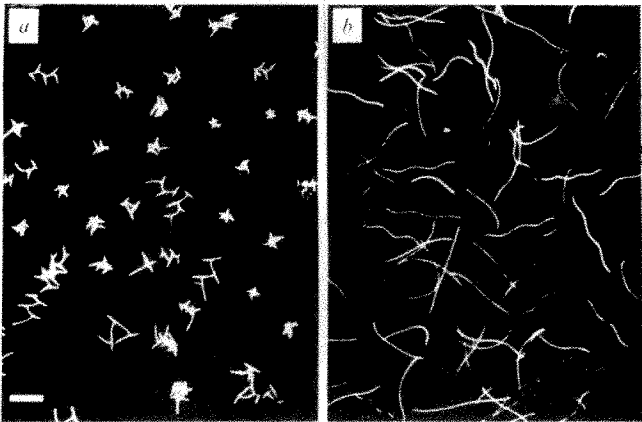


Table 2 Cross breeding analysis between wild type and beavioural mutants in *P. caudatum*

Cross	Generation	Phenotype		χ^2 and <i>P</i>	Survival (%)
		mutant	wild type		
13B × Kyky 1	F ₁	0	85	$\chi^2 = 0.01$ <i>P</i> = 0.9–0.95	60.7
	F ₂ Observed	4	15		22.6
	Expected	4.8	14.2		
11C × Kyky 1	F ₁	0	28	$\chi^2 = 0.10$ <i>P</i> = 0.7–0.8	20.0
	F ₂ Observed	10	25		27.6
	Expected	8.8	26.2		
13D × Kyk201	F ₁ Observed	11	17	$\chi^2 = 0.64$ <i>P</i> = 0.3–0.5	66.7
	Expected	14	14		

potential, which, as expected, was not followed by a regenerative Ca action potential of the general membrane (Fig. 2g). Weak reversal was observable only in the cilia on the mechanically stimulated anterior membrane. In wild type cells a regenerative Ca action potential was always accompanied by an anterior mechanoreceptor potential (Fig. 2e), caused by an increase in Ca²⁺ permeability on mechanical stimulation²². Thus the mechanosensitive Ca gating system in the anterior membrane is functional in CNR cells. Electrogenetic malfunction in the membrane of CNR is restricted to its voltage sensitive Ca gating system.

Mutant 13D showed remarkably long-lasting backward swimming, due to ciliary reversal, when transferred to K⁺-rich medium (about five times as long as for the wild type, Table 1). The resting membrane potential of 13D cells was much more depolarised than that of the wild type (about twice as much)

by an increase in the external K⁺ concentration, especially in the lower concentration range (0.5–4.0 mM). The resting membrane of 13D therefore has a higher K⁺ permeability than the wild type²³. We shall call 13D specimens 'K⁺-sensitive'. K⁺-sensitive showed a more marked increase in K⁺ permeability than wild type in response to an inward current. This was demonstrated in a phase plane trajectory as a shift in the membrane e.m.f. toward the K⁺ equilibrium potential (hyperpolarising direction) when the inward current stopped (compare Fig. 3h and c). Electrical responses of K⁺-sensitive to an outward current were essentially similar to those of the wild type (Fig. 3a and g).

Mutant 11C was distinctive for its slow swimming speed, being about half as rapid as the wild type (Table 1). Forward swimming of ATP-Mg²⁺-reactivated Triton-extracted cells of 11C was significantly slower than that of the wild type (171.2 ± 10.6 μm s⁻¹ in 11C, 234.5 ± 17.2 μm s⁻¹ in the wild type at 25 °C)^{24,25}. This, together with the fact that the electrophysiological characteristics of the specimen were essentially similar to those of the wild type, indicates that the slower swimming of 11C is due primarily to some malfunction in its ciliary motile system. We shall call 11C 'slow swimmer'.

We investigated gene differences between the mutants and the wild type by cross breeding. As Table 2 shows, when CNR or slow swimmer was crossed to a wild type (Kyky 1), all F₁ progeny had wild type behavioural characteristics. Phenotypic segregation occurred in F₂ clones with an expected ratio of 1:3. Therefore the abnormal behaviour of CNR and slow swimmer is under the control of a single recessive gene²⁶, designated *cnrA* and *sl* respectively. In the case of K⁺-sensitive, the F₁ segregated phenotypically into those with K⁺-sensitive characteristics and those with wild type characteristics in a ratio of 1:1. Therefore we consider that the high K⁺ sensitivity of K⁺-sensitive specimens is controlled by a dominant gene, K⁺S.

These mutants were isolated in R. Eckert's Laboratory at the University of California, Los Angeles, with the support by grants from the US PHS and NSF. The work was also supported by grants from Mitsubishi foundation and the Ministry of Education of Japan. We thank R. Eckert, S. Hagiwara, C. Kung and K. Hiwatashi for valuable discussions. We also thank M. Knutson for technical assistance and D. Fujii for help with the manuscript.

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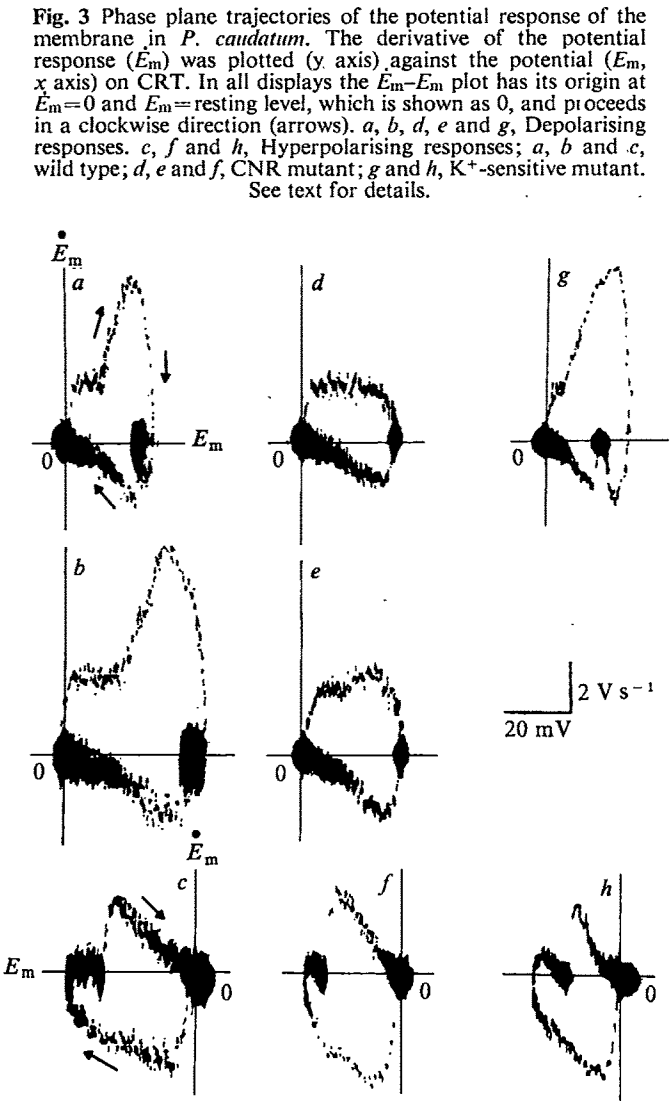
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Received 18 October 1977; accepted 3 January 1978.

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Scanning transmission electron microscopy of unstained biological sections

THE scanning transmission electron microscope (STEM) has made possible the direct visualisation of single heavy atoms¹ and, by exploitation of the high dark-field detection efficiency, has been used successfully for the imaging of stained and unstained biological specimens². We report here some preliminary results which indicate that STEM can also be utilised for the imaging of unstained biological sections.

The use of STEM for biological electron microscopy has several advantages over conventional transmission electron microscopy. The irradiation dose is easily controlled by shifting the scanning raster to an adjacent area for focusing and stigmation and by the use of image storage devices (scan converters) and beam blanking. Simultaneous use of several imaging modes (for example, bright- and dark-field) combined with analogue or digital image-processing techniques can be used to enhance 'on-line' the contrast of difficult biological specimens as described in this paper. Additionally, micro-analysis of easily identified specimen areas can be carried out *in situ* by energy-dispersive X-ray spectroscopy or electron energy-loss spectroscopy.

The material we have used for this work has been sectioned rigor flight muscle from *Lethocerus cordofanus*. This material was chosen because it has well defined structure, recognisable at both low magnification (the sarcomere repeat) and at higher magnification (the actin and myosin filaments and cross-bridges), and because an objective assessment of the image resolution can be made by the use of optical diffraction. The muscle was fixed with glutaraldehyde and embedded in araldite as described previously³, except that the osmium fixation and staining were omitted.

The microscopy was carried out on a V.G. Microscopes HB5 STEM operating at 100 kV with a cold field-emission source, an annular dark-field detector and a magnetic sector electron spectrometer as bright-field detector. Images were recorded directly from the photographic display screen on to 35 mm film which could be used without further processing for optical diffraction measurements. In order to minimise specimen dosage at high magnification, the microscope was used in a 'single shot' manner, that is, the image from a single scan at lower magnification was stored on a scan converter and redisplayed, with the electron beam blanked, in order to select a suitable area of specimen. The magnification was then increased and an area immediately adjacent to the selected region used for focusing. Finally, the scan raster position was shifted over to the appropriate position and the image recorded 'blindly' on film and simultaneously on the scan converter. The scan converter image was then redisplayed to assess various parameters, including the quality and correctness of focus of the recorded image. The total electron dose at the specimen was measured with a Faraday cage fitted directly above the specimen position.

To obtain sufficient contrast from a specimen where the contrast producing mechanism is the small difference in electron scattering between protein and the surrounding matrix of

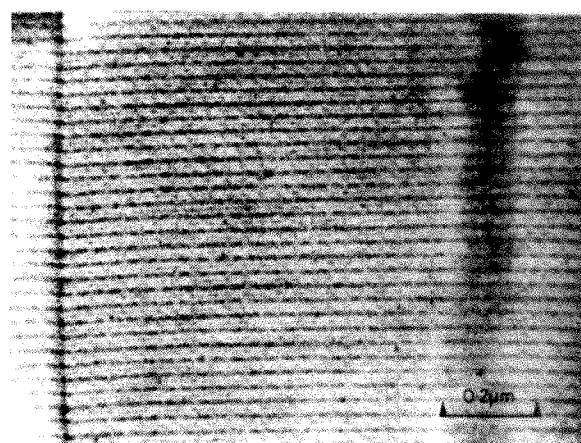


Fig. 1 STEM micrograph of unstained insect flight muscle section, original magnification 50,000 \times . This is a mixed-signal bright-field image, so that electron dense material appears dark. The central vertical dark region is the M band, the less dense vertical region to the right, the Z band. Scale bar, 0.2 μ m.

embedding material, and to obtain an acceptable signal-to-noise ratio, it was necessary to use a mixed-signal mode of imaging. This had earlier been proposed for heavy atom imaging¹ and has been used for visualisation of unstained protein in ferritin particles⁴. The microscope was adjusted to give equal simultaneous signals in the dark-field and incoherent bright-field modes of operation. The two signals were then mixed in the algebraic mode $(BF - DF)/(BF + DF)$, and the mixed signal used to produce the final image. The resulting mixed signal has higher contrast for a given signal-to-noise ratio than either signal taken alone, and has the advantage that noise introduced by fluctuations in the electron beam is cancelled out by the division.

A typical image of unstained insect flight muscle is shown in Fig. 1. This was recorded with a 'single shot' after focusing on an adjacent area as described above, with a total electron dose, at an instrument magnification of 50,000 \times , of less than 25 electrons \AA^{-2} . The appearance of the thick and thin filaments is similar to that seen in stained sections and the cross-bridge structure can be seen weakly. The appearance of the M and Z bands is, however, different. The M band appears much darker than in stained sections, whereas the Z band, which stains very heavily, here appears to have a similar average density to that of the rest of the sarcomere. The appearance of the Z band was, in fact, somewhat variable, often being a little denser than the surrounding sarcomeres, but never appearing as dense as when seen in stained sections. It is not possible to say what these

Fig. 2 STEM micrograph, taken at magnification 100,000 \times . The dark region to the right is the M band. At this magnification, thick and thin filaments, and cross-bridges are visible. The narrow dark line to the left is the boundary of the previous scan. Loss of material has taken place to the left of this line, causing a distortion of the filament pattern.



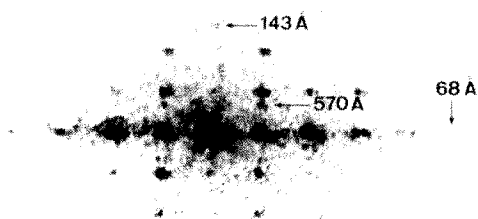


Fig. 3 Optical diffraction pattern taken from the image of Fig. 2. (The pattern has been rotated through 90° relative to the image.) There are strong reflections along the 380 Å and 190 Å layer lines, the 540 Å layer line is also clearly visible, and the 140 Å meridional reflections are weakly present.

density differences mean in terms of material present, but an attempt is being made to quantify the image density-mass thickness relationship.

Figure 2 shows an image taken at $100,000\times$ magnification, again by focusing and adjusting instrumental astigmatism off the area to be imaged. One of the main difficulties in working with unstained material is the very rapid loss of material (etching) which takes place during each scan. At the boundary between the two scans, there is a marked distortion of the filament pattern and a lower density in the region to the left that has been exposed to two consecutive scans. Some variation in density can be seen along the thin filaments, and cross-bridges can be distinguished quite clearly. An optical diffraction pattern of this image is shown in Fig. 3. The resolution in the equatorial direction extends to about 65 Å and in the meridional direction to about 148 Å. The overall resolution is close to that obtained for stained sections, and weaker intermediate layer lines⁵ were visible. The images shown in Figs 1 and 2 were of sections in the thickness range 600–800 Å. Work in progress on other materials indicates that good contrast can also be obtained for thinner sections.

These results indicate that for unstained biological material, conventionally fixed and embedded, resolutions approaching those obtained for stained sections (that is, the limit imposed by the preparative technique itself) can be obtained by STEM mixed-signal imaging. It is hoped that resolutions may be extended by improved specimen preparation methods, including cryo-techniques⁶, or in the case of periodic structures, by using low-dose imaging⁷. A further important advantage of the ability to image unstained material is the combination of this form of imaging with the use of STEM for X-ray microanalysis. Preliminary results indicate that secondary X-ray detection with a sensitivity at least a factor of 10 higher than that obtainable by conventional X-ray dispersive systems, and probe sizes down to a few angstrom units in diameter, is possible. The two techniques used together will provide a powerful tool for the characterisation of elemental distribution in biological materials.

We thank Mrs B. M. Luke and B. Bullard, Department of Zoology, Oxford University, for providing the insect muscle sections.

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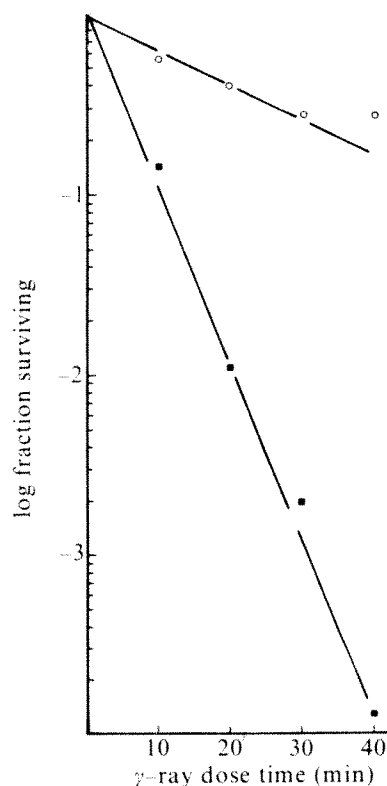
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Cellular glutathione is a key to the oxygen effect in radiation damage

OXYGEN is known to enhance biological changes induced by ionising radiation^{1,2}. The changes include chromosome breaks in *Tradescantia*³, mutation production in *Drosophila*, maize and bacteria^{3–6}, and rate of mitosis in grasshopper neuroblasts⁷. The biological and physical bases for the various enhancements remain obscure, and it has not yet been established that they are the result of a common mechanism. The quantitative aspects of the relationship between oxygen concentration and radiation response have been measured: for example, killing of *Escherichia coli* by X-ray radiation at a constant radiation dose⁸ and various interpretations of the quantitative aspects of the response base have been made^{9–12}. Ionising radiation has been proposed to interact with water to produce several products and these products interact with cellular material to produce the biological effects observed. In the reactions proposed, cellular sulphhydryl groups are supposed to be a primary cellular constituent which is reactive with the radiation products from water. We report here that one major cellular sulphhydryl constituent, glutathione (GSH), is apparently the major component in the interaction between radiation products and the cell, for cells unable to synthesise glutathione cannot be protected against killing by ionising radiation by reduction of the external oxygen concentration.

The response of GSH⁺ *E. coli* strain to irradiation in cellular suspension bubbled with air and argon (99.999% pure) is shown in Fig. 1. The isoefficiency dose ratio, that is, that dose ratio that gives equivalent survival in air and in the absence of oxygen⁹—now probably better known as oxygen enhancement ratio (OER), is about a factor of three. That is, in the presence of oxygen, ionising radiation is

Fig. 1 The oxygen effect with a GSH⁺ *E. coli* strain. ■, Survival in cell suspension bubbled in air; ○, survival of suspension bubbled for 10 min with argon. Cells grown in Penassay broth, washed, and irradiated in phosphate buffer, pH 6.9. Dose rate 2,230 rad min⁻¹ from a ⁶⁰Co source. Post-irradiation plating incubated at 30 °C.



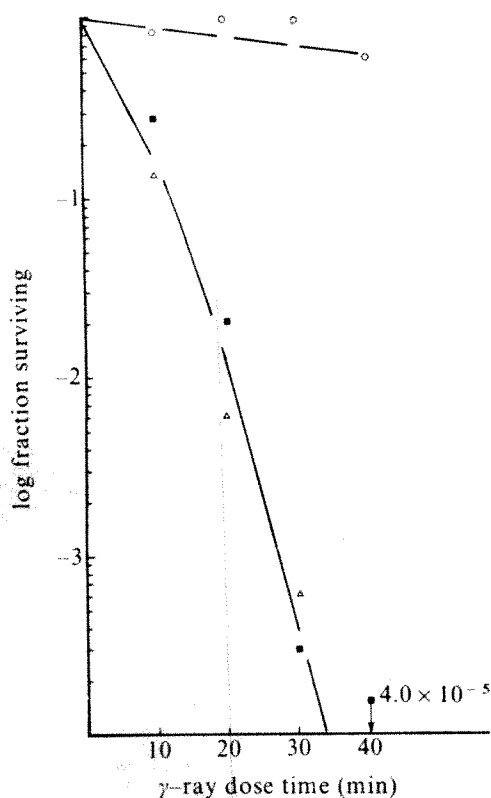


Fig. 2 The lack of a protective effect of argon treatment (oxygen removal) on a GSH⁻ *E. coli* mutant¹³. ○, GSH⁺ strain bubbled with argon; ■, GSH⁻ strain bubbled with argon; △, GSH⁻ strain bubbled with air. In this experiment, a spectinomycin-resistant derivative (20 µg ml⁻¹ spectinomycin) was selected in the GSH⁻ strain, and cells from a culture of it mixed with its spectinomycin-sensitive parent in buffer and irradiated. Plating on media with and without spectinomycin permits discrimination. Incubation at 30 °C. Dose rate, 2,230 rad min⁻¹.

about three times more effective than in its absence, or near absence.

A glutathione deficient mutant (GSH⁻)¹³ does not show the increased survival of its parent in the absence of oxygen (Fig. 2) and it is presumed that the response is related to its inability to synthesise glutathione. It should be noted that in the experiment of Fig. 2, a spectinomycin-resistant derivative mutant of the GSH⁻ strain was used in mixed suspension with its wild-type GSH⁺ parent: the mixed suspension irradiated and plated on media (±spectinomycin) permits counting of both GSH⁺ and GSH⁻ strains irradiated in the same suspension and in identical irradiation conditions.

The spectinomycin resistance of the GSH⁻ strain is not responsible for the radiation response, for its spectinomycin sensitive parent is not made more resistant to radiation by reduction of external cellular oxygen (data not shown).

Five experiments on the GSH⁻ mutant all gave the same result and clearly show that the GSH⁻ *E. coli* cells cannot be protected against radiation by oxygen removal.

A reversion of the GSH⁻ strain was selected on the basis of faster growth rate in broth than its parent, and the glutathione content of it, the parent, and the wild type measured by a modification of the procedure of Fuchs and Warner^{13,15}. The glutathione contents were (nmol per mg protein): GSH⁺, 17.6; GSH⁻, 0.27; the GSH⁺ reversion, 10.1.

The GSH⁺ reversion was irradiated in the presence of air and in suspension bubbled with argon to remove oxygen. The results of this experiment are shown in Fig. 3. Clearly, the reversion shows a response qualitatively similar to the wild type, and quantitatively, based on its GSH content, to irradiation in reduced oxygen.

The mechanism by which cellular glutathione protects against ionising radiation damage in the presence of oxygen

is not known. A simple question can be dealt with, however. Can growth of the GSH⁻ deficient mutant in the presence of glutathione protect the cells against radiation damage under reduced oxygen tension? The results indicate that this is so; GSH⁻ mutant cells grown in glutathione, washed and resuspended in buffer are consistently more resistant to radiation under reduced oxygen tension: 3,100-fold; 44-fold; 41-fold; 100-fold; 10-fold; 8-fold in six separate experiments. Some variable phenotypic reversal can be achieved.

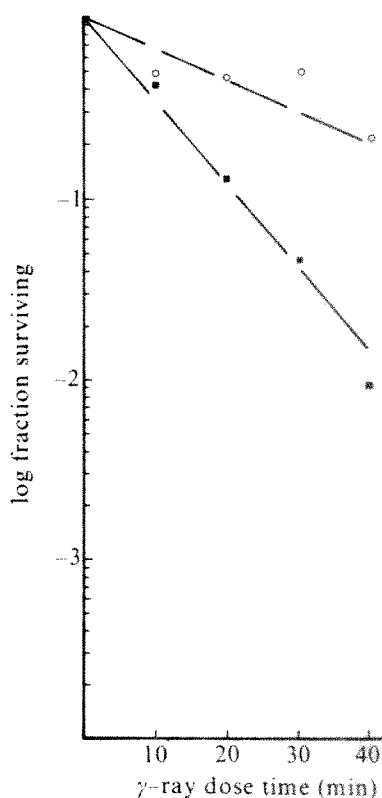
Indirect effects of ionising radiation have been reviewed^{16,17}, and recent summaries of substances that sensitise to radiation presented^{18,19}. Quantitative relationships between radiation response and oxygen tension have been considered linear¹², but this assumption is probably not correct¹¹. Despite these studies, the biochemical basis for the oxygen effect remains obscure.

It has been speculated that the oxygen effect is related to cellular sulphhydryl groups because of the reactive nature of these groups with oxidising radicals produced by irradiation of water. A horizontal study of a number of bacterial strains, attempting to relate radiation resistance to GSH content has been reported²¹. The results presented above limit this view to the oxygen effect. In addition, since total cellular GSH groups are not reduced in GSH⁻ mutants^{13,14}, it suggests specificity to GSH and not sulphhydryl groups *per se*.

The nature of this specificity remains to be determined, and it is not known in what manner GSH participates in cell protection, or at what stage in the radiation process it is needed. Growth in the presence of GSH seems to partially reverse the phenotype of the GSH⁻ mutant, and these experiments need to be expanded as well as a study of the effects of post-radiation addition of GSH.

Methyl glyoxal and diamide [diazene dicarboxylic acid, bis(*N,N*-dimethylamide)] have been shown to reverse the

Fig. 3 Survival of a spontaneous GSH⁺ reversion of the GSH⁻ strain. ■, Bubbled with air; ○, bubbled with argon. Cell growth, preparation and irradiation of cells as in Figs 1 and 2. Reversion to GSH⁺ has restored qualitatively the response to oxygen removal, and almost quantitatively the amount of resistance, since the reversion has only about 50% the GSH content of the wild type. Compare with Fig. 1.



protective effect of anoxia^{18,20}. Since these compounds are reagents reactive with sulphhydryl groups, perhaps specifically with GSH, the mechanism by which they act can now be understood. Presumably, destruction of cellular GSH by these reagents makes cells phenotypically identical to the GSH⁻ mutant.

This research was supported by USNSF grant BMS 7507343. We thank Drs Fuchs and Warner, and Apontoweil and Berends for supplying bacterial strains.

Note added in proof: Since the above experiments were conducted, we have received a γ -glutamyl cysteine synthetase mutant, and an independent glutathione synthetase mutant from Apontoweil and Berends¹⁴. Both of these strains cannot be protected against ionising radiation damage by oxygen removal.

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Parallel induction of sister chromatid exchanges and infectious virus from SV40-transformed cells by alkylating agents

ALTHOUGH little is known about the biochemistry of the formation of sister chromatid exchanges (SCEs), it has been suggested^{1–3} that they reflect some aspect of cellular DNA repair. Because agents that promote SCE formation (for example, refs 2, 3 and 6–9) also induce infectious virus from certain SV40-transformed hamster cell lines^{10,11} and because evidence suggests that virus induction involves DNA repair^{12,13}, we have investigated a possible correlation between virus induction and SCE formation. We tested both events in the same cells in identical conditions. Parallel dose-response curves for both SCE formation and induction of infectious SV40 virus were observed with two alkylating agents, mitomycin C and ethyl methane sulphonate (EMS), which required effective concentrations differing by a factor of 10,000.

We used four clones of SV40-transformed hamster kidney cells, differing in their capacity to produce infectious virus¹¹. Clone A cells and to a lesser extent clone E cells release virus spontaneously. In both cell lines, as well as in clone B cells, which have not been found to produce virus spontaneously, virus induction can be stimulated as much as 10^3 – 10^4 -fold by exogenous agents such as mitomycin C¹¹. Clone G is a non-producing, non-inducible cell line while BHK-21 is an untransformed hamster kidney cell

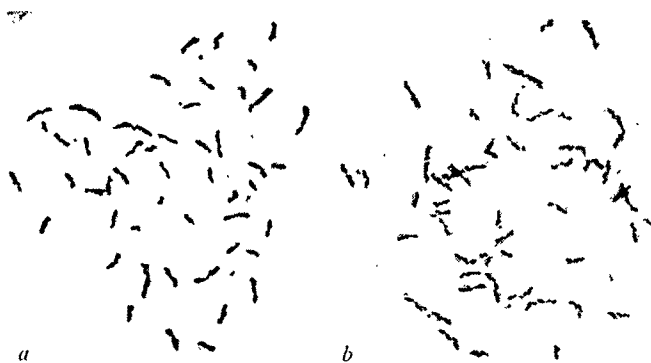


Fig. 1 Detection of SCEs and their induction by mitomycin C in hamster metaphase chromosomes by BRdU-33258 Hoechst-Giemsa methodology²¹. SV40-transformed hamster kidney cells (clone G) were grown for 24 h in medium (Eagle's minimal essential medium (Gibco) with four times the usual concentration of vitamins and essential amino acids (MEM $\times 4$), 10% foetal calf serum (Gibco), 2 mM glutamine, penicillin (250 U ml⁻¹, and streptomycin sulphate (250 μ g ml⁻¹) containing 2.5×10^{-5} M BRdU. Cell collection and slide preparation using 33258 Hoechst dye and Giemsa stain were as before^{22,23}, except that: *a*, a high concentration of colcemid (Calbiochem; final concentration 8 μ g ml⁻¹) was added for the final 6 h as the control; *b*, mitomycin C (0.03 μ g ml⁻¹) was added 18 h before cell collection.

line which was used as a control. Duplicate samples of cell cultures were grown in the presence of BRdU, exposed to alkylating agents, and, at appropriate times, assayed either for SCE formation or production of infectious virus.

As previously described for other cell types, SCEs were detectable as reciprocal alterations in staining intensity along metaphase chromosomes of hamster cells which had incorporated BRdU for two divisions (Fig. 1*a*). An average of 11 SCEs was observed per metaphase in uninduced clone G cells. Treatment with mitomycin C (0.03 μ g ml⁻¹) increased the average frequency of SCEs in these cells to 88 per metaphase (Fig. 1*b*).

The average frequency of SCE formation, normalised per chromosome number of each cell line, increased in all lines in proportion to the dose of either inducing agent (Figs 2*a*, *b*). Mitomycin C was effective in inducing SCEs at much lower concentrations than was EMS. For example, a fourfold increase in SCEs over baseline frequency was caused by mitomycin C, in a concentration of less than 20 μ g ml⁻¹ but required EMS at approximately 300 μ g ml⁻¹. Similar frequencies of baseline and induced SCEs were observed for all cell lines. Chromosomal aberrations increased with increasing doses of inducing agent, averaging 3–4% of the number of SCEs. Aberrations were not always detected at the lowest concentrations of inducing agent, whereas SCEs were always measurable and are therefore more sensitive indices of response to the agents.

Virus yields paralleled SCE frequency in their response to increasing doses of mitomycin C or EMS (Figs 2*c*, *d*). Absolute levels of virus production were significantly different in clones A, E and B, although the relative responses of the cells to mitomycin C and EMS were similar. Cell survival, as measured by colony-forming ability, was approximately the same for each virus-producing cell line within the concentration ranges used of either agent.

Interclonal differences in absolute virus yields could depend on several factors, such as the nature or sites of viral genome integration, completeness of the integrated genome or mechanisms controlling viral genome excision, replication, transcription or post-transcriptional events. Whatever the explanation, all virus producers showed an induction response which paralleled the SCE increase.

All experiments were performed in the presence of

BRdU, which can induce SCEs^{2,6,8} and certain DNA and RNA viruses in normal and/or transformed cells¹⁴⁻¹⁷. In the hamster clones used in this study BRdU increased the absolute yield of SV40 two to fivefold but had little effect on the dose-dependent response to alkylating agents. The details of BRdU effects on DNA and RNA virus induction are under investigation.

The mechanisms of virus induction and SCE formation may involve DNA repair with some enzymatic features in common. DNA breakage seems to be associated with virus activation from transformed cells containing integrated viral genomes¹¹. Caffeine, which causes the accumulation of gaps in DNA damaged by ultraviolet light in rodent cells, by inhibiting post-replication repair, stimulates the induction of SV40¹³ and SCE¹⁸ by ultraviolet light. It might be the persistence of gaps which results in this enhancement. Pathways of DNA repair, perhaps related to recombinational events and analogous to SOS functions in prokaryotes¹⁹ and possibly in eukaryotes²⁰, may be involved in SCE formation. An association of SCE formation with an error-prone repair process could account for the observed correlation between SCEs, mutagens and carcinogens.

The exact relationship of SCE formation and virus induction remains to be determined, although the two

events show a striking correlation. Further examination of their temporal connection and biochemical characteristics in relation to cellular DNA repair processes may help to explain their biological significance. In any case virus induction is a highly sensitive biological marker for further studies on the mechanism(s) of DNA repair and SCE formation in mammalian cells.

We thank Lawrence F. Kleinman and William B. Rogers for technical assistance. This research was supported by grants from NIH and the American Cancer Society. J.C.K. and S.A.L. are recipients of Research Career Development Awards from NIH. 33258 Hoechst was given by Dr H. Loewe, Hoechst AG, Frankfurt.

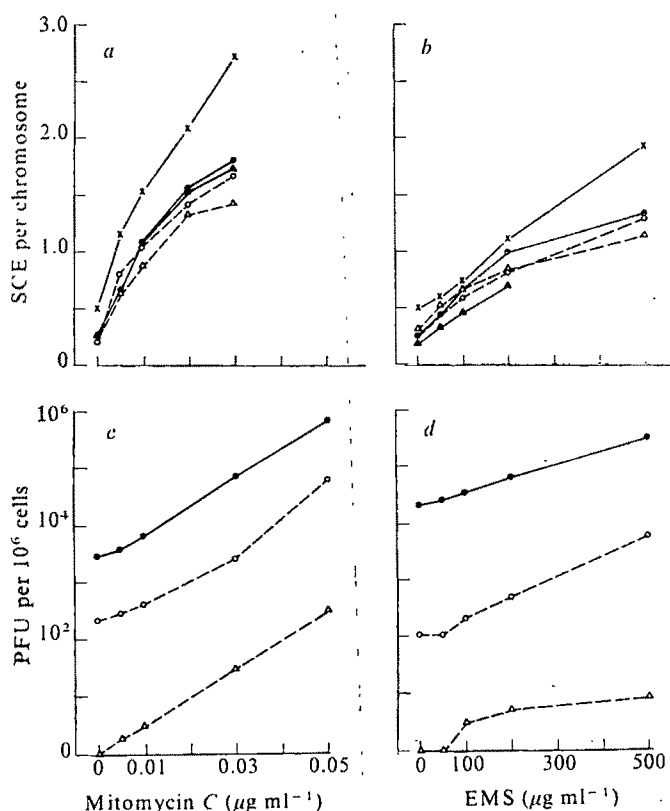
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Fig. 2 Induction of SCEs and infectious virus in different clones of SV40-transformed hamster kidney cells by mitomycin C or EMS. Cells were cultured for two divisions in growth medium containing 2.5×10^{-5} M BRdU. Eighteen hours before collection for SCEs, either mitomycin C or EMS was added to the culture medium. Cell collection and slide preparation were as described for Fig. 1. SCE frequencies are expressed per chromosome (*a* and *b*). ●, Clone A; ○, E; △, B; ▲, G; X, untransformed BHK-21 hamster cells. Replicate cultures for assay of infectious virus production were treated as above except that mitomycin C or EMS was removed 24 h after addition to the cultures. Cells were washed with phosphate-buffered saline and fresh growth medium was added before the cultures were reincubated at 37 °C for 72 h. Procedures for collection, preparation of cell-free extracts and plaque assay for SV40 virus infectivity were as described¹¹. Virus yields are expressed as plaque-forming units (PFU) per 10^6 cells present at the time of addition of the inducing agent (*c* and *d*). ●, Clones A; ○, E; △, B.



Received 27 September; accepted 19 December 1977.

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Induction of autoreactive T lymphocytes and their suppressor cells by cyclophosphamide

THERE is increasing evidence of potentially autoreactive cells in normal animals^{1,2}. Expression of autoimmunity, however, may not arise as a primary effector cell abnormality but more possibly as the consequence of a defect in a thymus-dependent control mechanism³⁻⁵. Cyclophosphamide (CY) enhances the capacity of mice to give T cell-dependent responses, such as delayed type hypersensitivity (DTH) induced by sheep red blood cells⁶. It was suggested that B cells were the targets of CY action⁷ and that the enhanced DTH reaction was due to inhibition of antibody production and consequently to lack of the antigen-antibody complexes responsible for inhibition of DTH. Askenase *et al.*⁸ presented evidence for an alternative explanation for enhancement of DTH by CY. They demonstrated augmented DTH to sheep red blood cells with doses of CY which did not influence antibody responses, suggesting the existence of CY-sensitive suppressor cells. Similar conclusions

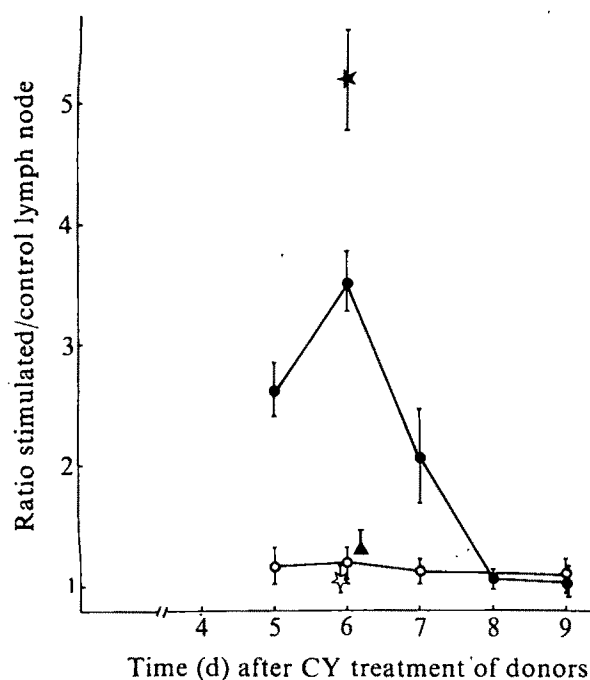


Fig. 1 Local GvH reaction in syngeneic host. Female BALB/c or AKR mice, 6–8-weeks old, were injected intraperitoneally with 125 mg kg⁻¹ cyclophosphamide (CY). Spleen cells were collected at various intervals. Cells from 6–12 spleens were pooled, suspended in Hanks BSS (supplemented with 5% foetal calf serum, 100 units ml⁻¹ penicillin, 100 µg ml⁻¹ streptomycin) and passed through a nylon wool column¹⁰. The eluted cells were washed three times with serum-free medium. As tested on cell smears stained with either Giemsa or with fluorescein-conjugated rabbit anti-mouse immunoglobulin, the nylon wool filtered cells were depleted on both phagocytic cells (less than 0.1%) as well as on B lymphocytes (less than 5%). In some experiments, nylon wool-filtered AKR spleen cells were incubated with anti-theta serum (C3H anti-AKR Thy-1.1 from Searle Diagnostic, High Wycombe, England) and guinea pig complement. Control cells were incubated with normal C3H serum and complement. After incubation, the cells were washed three times with serum-free medium. 3×10^6 cells in a volume of 0.05 ml were injected subcutaneously into the right hind footpad of a syngeneic recipient. Six days later, the draining and the contralateral lymph nodes were removed and weighed. The results are expressed as the ratio of the weights of stimulated to contralateral unstimulated popliteal lymph node. ●, Cells derived from CY-treated BALB/c donors. ○, Cells derived from untreated BALB/c controls. ★, Cells derived from CY-treated AKR donors and injected into AKR recipients. ▲, Cells derived from untreated AKR controls. ▲, Cells derived from CY-treated AKR donors after anti-theta treatment. Each value represents the mean \pm s.e.m. obtained from 5–7 recipients.

have been drawn by Röellinshoff *et al.*⁹, who observed induction of cytotoxic lymphocytes in mice to hapten-conjugated syngeneic cells after treatment with CY. We report here that injection of normal mice with CY results in manifestation of autoreactive T lymphocytes followed by the appearance of suppressor cells counteracting autoreactivity.

Balb/c or AKR mice were injected with a single dose of CY (125 mg kg⁻¹). At various time intervals splenic T cells (prepared by passing the cells through nylon-wool column¹⁰) were injected subcutaneously into the footpads of syngeneic recipients, and the resulting local graft versus host (GvH) reaction^{2,11} was determined in the draining lymph nodes. Autoreactive cells could be detected in the spleens of donors with a peak 6 d after CY treatment (Fig. 1). Autoreactive cells treated with anti-theta serum and complement failed to induce a GvH reaction. As in local GvH reactions induced by allogeneic cells^{12,13}, the GvH reaction in our syngeneic system depends on cells of host origin. This was shown by failure of X-irradiated (800 r) or CY-treated (125 mg kg⁻¹) recipients to give a GvH reaction when injected with autoreactive cells 1 d after this procedure. T cells derived from donors 8–9 d after CY treatment did not elicit a GvH reaction. Moreover, these cells, mixed with autoreactive cells, abolished the GvH reaction (Table 1), but if the donors were intravenously injected 20 h after CY with 5×10^7 syngeneic thymocytes autoreactive cells were not detectable. Splenic T cells of these animals collected 5 d after thymocyte injection suppressed the activity of autoreactive cells (data not shown), indicating CY-sensitivity of suppressor cell precursors.

It has been shown that T cells of neonatally⁴ or adult⁵ thymectomised mice injected into syngeneic recipients induce GvH reactions. The authors suggest that this phenomenon is due either to impaired thymus functions or loss of T-suppressor cells. This implies the coexistence of committed autoreactive and suppressor cells in normal spleen cell populations. Because suppressor cells are CY-sensitive, our results could be interpreted simply as being due to loss of suppressor cells, permitting detection of autoreactive cells; but the findings that normal spleen cells failed to inhibit expression of autoreactivity (Table 1) and that autoreactive cells do induce GvH reactions in normal syngeneic mice, argue against pre-existence of already committed suppressor cells. One of the crucial questions concerns the nature of self antigens which are recognised by the cells involved in this autoimmune phenomenon. Do autoreactive lymphocytes recognise normal or altered self-components, and what is the nature of the alteration? As previously shown¹⁴, mice injected with 125 mg kg⁻¹ CY are depleted (1–4 d after this treatment) of B cells and of subsets of T cells. The damage to the cells in the spleen is followed by a burst of rapidly proliferating B cells. B cell mitogens have been

Table 1 Demonstration of autoreactive lymphocytes and their suppression by regulatory cells

Treatment of donors	Nylon wool	No. of cells transferred	No. of recipients	Local GvH response lymph node ratio	
				Weight \pm s.e.m.	Cell number
none	no	3×10^6	10	1.18 ± 0.08	1.28
none	yes	3×10^6	16	1.16 ± 0.09	1.14
a	no	6×10^6	6	3.01 ± 0.32	3.80
a	yes	3×10^6	20	3.23 ± 0.21	4.43
b	yes	3×10^6	11	1.05 ± 0.08	1.10
c	yes	3×10^6	5	1.33 ± 0.37	1.69
d cells from a	yes	3×10^6			
+ normal spleen cells	yes	3×10^6	6	3.11 ± 0.30	4.09
d cells from a	yes	3×10^6			
+ cells from b	yes	1.5×10^6	6	1.38 ± 0.15	1.45
d cells from a	yes	3×10^6			
+ cells from b	yes	3×10^6	6	1.22 ± 0.11	1.07

Spleen cells were obtained from BALB/c donors as described in Fig. 1, for a, 6 d and b, 8 d after CY treatment; c, CY-treated donors were intravenously injected 20 h later with 5×10^7 normal thymocytes, spleens were removed 6 d after CY d, cells were mixed and injected in a volume of 0.05 ml into the hind footpad of the recipients. The results of the local GvH response are either calculated as the ratio in the weights \pm s.e.m. or as the ratio in the cell content between the stimulated and contralateral unstimulated lymph nodes 6 d after cell transfer. The ratios for the number of cells were obtained from pooled lymph nodes in each experimental group.

shown^{15,16} to induce the appearance of endogenous C-type RNA viruses in spleen cell culture supernatants and at the surface of splenic B cells. Therefore, it is possible that the 'autoreactive' T cells may recognise altered self antigens (possibly virus-altered) on proliferating B cells. This is supported by the findings that AKR mice, known to contain genetically much higher titres of endogenous ecotropic and xenotropic viruses¹⁷, when injected with CY, develop a greatly increased number of autoreactive cells, as compared to BALB/c mice.

The results indicate that CY treatment induces the appearance of 'new' antigenic sites (perhaps present at the surface of primitive B cells or as altered self-components due to endogenous virus induction) and the transient depletion of the spleen on T-suppressor cells. Both effects acting together permit manifestation of 'autoreactive' cells in normal animals.

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Received 19 July; accepted 14 December 1977.

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Is there a circulating human thymic factor that induces cyclic AMP synthesis?

THE action of factors which have been extracted from the thymus or found circulating in peripheral blood and which are active in *in vitro* lymphocyte differentiation assays¹ has been extensively studied. Astaldi *et al.*² have described a factor (SF) of molecular weight (MW) less than 50,000 present in the serum of normal donors; a thymic site of production was suggested because it was not found in serum from athymic donors. It also significantly elevated cyclic AMP levels in mouse thymocytes. Because of the potential significance of this finding to the study of thymic factor action, we have attempted to corroborate these findings but, as reported here, we have observed that a serum fraction from both normal and athymic individuals can raise cyclic AMP levels in mouse thymocytes. Neither serum fraction, however, produced the degree of stimulation of cyclic AMP levels described by Astaldi *et al.*². Increases in cyclic AMP were much lower than those observed using the β -adrenergic agonist isoproterenol. We are therefore unable to confirm the existence of a circulating thymic factor in humans which raises cyclic AMP levels in mouse thymocytes.

Sera were initially obtained from two normal donors and two myasthenia gravis patients who had undergone thymectomy (samples by kind permission of Dr J. Posner, Memorial Hospital and Dr G. Jenkins, Mt Sinai Hospital, New York City). As described by Astaldi *et al.*², we separated the fresh serum into two fractions using Amicon CF50 filters. The fraction passing through the filter containing the SF (MW < 50,000)

was employed in the present studies. The fractionated SF samples were stored in aliquots at -20°C . Serum fractions from normal individuals were used after being stored for two days and fractions from athymic patients were used after two weeks' storage. As reported by Astaldi *et al.*², we observed that the serum fraction retained by the filter (MW > 50,000) was inactive in the assay used.

SF samples were evaluated for their ability to increase cyclic AMP levels of mouse thymocytes. Incubations were terminated either by rapid freezing and assayed for cyclic AMP *in situ* according to the procedure of Astaldi *et al.* ('unpurified' sample), or terminated by trichloroacetic acid precipitation followed by ether extraction and Dowex-1 column purification before assaying for cyclic AMP (ref. 3) ('purified' sample). Because the SF samples contained cyclic AMP (up to 50% of the total), the cyclic AMP levels of the serum were subtracted from the total cyclic AMP measured in the samples containing both cells and the factor, thus giving the net value provided by the cells. Basal cellular levels of cyclic AMP in the unpurified samples were 1.7 pmol per 20×10^6 cells and 2.8 pmol per 20×10^6 cells in purified samples (Table 1). Total cyclic AMP levels were increased in a dose-dependent manner by both normal and athymic SF, but when the cyclic AMP component of the SF was subtracted, cellular increases were marginal and inconsistent in samples assayed by the procedure of Astaldi *et al.*². In purified samples, however, both normal and athymic SF's stimulated cyclic AMP levels in a dose-dependent fashion by up to two and a half times. These increases were small in comparison with the stimulatory effect of isoproterenol (Table 1 and refs 2, 4).

Manipulation of the thymocytes or of the SF's did not change their effects on cyclic AMP levels. Thus, serum fractions prepared from normal donors by rapid defibrination of whole blood followed by immediate transfer to an ice bath at 4°C produced no greater enhancement of cyclic AMP levels in thymocytes than those prepared by allowing whole blood to

Table 1 Effects of athymic and normal serum fractions on mouse thymocyte cyclic AMP levels

Addition		Unpurified sample		Purified sample	
		Total cyclic AMP	Net cyclic AMP	Total cyclic AMP	Net cyclic AMP
Normal SF	0	1.70	1.70	2.84	2.84
	0.125 ml	2.26	1.50	4.21	3.17
	0.25 ml	3.73	2.20	7.21	5.12
	0.50 ml	4.70	1.64	10.99	6.83
	0.25 ml	1.53	0	2.08	0
Athymic SF	0	1.70	1.70	2.84	2.84
	0.125 ml	2.96	2.28	4.89	3.97
	0.25 ml	3.56	2.20	6.21	4.36
	0.50 ml	4.99	2.27	10.36	6.66
	0.25 ml	1.36	0	1.85	0
10 ⁻⁵ M Isoproterenol		+		185.6	185.6

Serum fractions of less than 50,000 MW were prepared from two normal and two athymic donors as described by Astaldi *et al.*². Thymocytes were obtained from 6-10-week female C57BL/6 mice and resuspended in triplicate in Hank's Balanced Salt Solution without phenol red. Each tube contained 20×10^6 cells. 0.125, 0.25 or 0.5 ml aliquots of the serum fractions were added to make a total volume of 1 ml per tube. After incubating for 5 min at 37°C , the incubation was either terminated by rapid freezing and assayed for cyclic AMP *in situ* as previously described by Astaldi *et al.*², or terminated by trichloroacetic acid precipitation followed by ether extraction and Dowex-1 column purification before assaying for cyclic AMP (ref. 3). Aliquots of purified samples were tested with a phosphodiesterase preparation. >95% of the cyclic AMP measured was destroyed by this treatment, confirming the purity of the material. Results are expressed as pmol cyclic AMP measured per 20×10^6 cells. 'Total cyclic AMP' is the total amount of cyclic AMP measured, 'Net cyclic AMP' has the cyclic AMP content of the SF subtracted out, so that the values reflect only cell-produced cyclic AMP.

clot. Moreover, neither basal nor stimulated levels of cyclic AMP were affected by pretreating the thymocytes on nylon wool columns.

Table 1 shows that both total and net cellular cyclic AMP levels (basal and stimulated) are much higher after sample purification, suggesting that there may be factors in the unpurified material which interfere with the cyclic AMP assay. We have previously found that such factors must be removed by Dowex-1 chromatography before accurate cyclic nucleotide measurements can be made³. Phosphodiesterase treatment confirmed that our purified samples after Dowex chromatography were >95% cyclic AMP. It is possible that Astaldi *et al.* were not measuring true cyclic AMP levels, but rather some other as yet unknown contaminating material. All our subsequent data were obtained by isolating and purifying cyclic AMP from the sample after acid precipitation.

Our results differ from those of Astaldi *et al.*² in that their basal levels of cyclic AMP were higher than ours (10 compared to our 2.8 pmol per 20×10^6 cells). Our values are in close agreement with those obtained by others^{4,5}. Moreover, although we have also observed that stimulation with SF is dose-dependent, we have observed much lower stimulated levels and smaller net increase in cyclic AMP than did Astaldi *et al.* The reason for these discrepancies remains unknown. Differences may in part relate to the relative health of the cells employed, failure to calculate cyclic AMP levels in the SF sample and to subtract it from the total, and possible difficulties in measuring cyclic AMP in unpurified samples.

Our preliminary evidence also indicated that both normal and athymic serum fractions exhibited almost identical dose-dependent elevations of cyclic AMP levels in contrast to the findings of Astaldi *et al.*² whose serum fractions from athymic patients were without effect on cyclic AMP levels in thymocytes. We therefore prepared SF's from a further seven patients who had undergone thymectomy following treatment for myasthenia gravis (age range 26–54 yr; time post-thymectomy 3 months to 12 years 3 months). The effect on mouse thymocytes of the serum fractions from eight of the nine athymic patients is shown in Table 2, and compared with the effects of identical fractions prepared from three normal donors.

SF's were assayed either the day they were prepared or after being stored for 24 h at -20°C . Samples were stored in aliquots at -20°C and retested at intervals over a period of up to two months. Although variations in cyclic AMP elevation were observed over this period with individual SF's, there was no correlation between the level of cyclic AMP induced and sample storage time. It is apparent from Table 2 that the levels of cyclic AMP induced by serum fractions from athymic patients are almost identical to those induced by fractions from normal donors. SF from only one athymic patient failed to induce increases in cyclic AMP. Although the treatment of this patient was not appreciably different from that of the eight others, her clinical state was one of the most serious despite thymectomy. This suggests that lack of a thymus may not be central and that the patient may be deficient in some other circulating endocrine product which could raise cyclic AMP levels.

Table 2 Effects of serum fractions prepared from normal and athymic human donors on cyclic AMP levels in mouse thymocytes

Serum fraction volume	Normal	Athymic
0	1.00	1.00
0.125 ml	1.61 ± 0.27 (9)	1.76 ± 0.13 (12)
0.25 ml	2.16 ± 0.35 (9)	2.93 ± 0.29 (10)
0.50 ml	4.30 ± 2.04 (6)	4.66 ± 1.05 (5)

Results are expressed as stimulation with regard to control \pm s.e.m. (no. of separate determinations). Cyclic AMP present in each serum volume was subtracted from total cyclic AMP measured. Experimental conditions as in legend to Table 1. Basal levels of cyclic AMP; 3.6 ± 0.4 pmol per 20×10^6 cells.

Our observations are at variance with the findings of Astaldi *et al.*² who reported that serum fractions from 18 out of 18 athymic donors could not elevate cyclic AMP levels in mouse thymocytes. We suggest that a cyclic AMP-raising material is present in the serum and that the severity of illness rather than the absence of a thymus may determine whether the factor is present in serum. In any case, we consider it more probable that the factor is synthesised outside the thymus, and is in no way related to circulating thymic factors⁶ the activity of which has been shown to decrease with time after thymectomy in experimental animals. Several circulating products, such as prostaglandins or adrenaline, with known cyclic AMP-raising properties in lymphocytes, may be likely candidates and defects in the secretion of one of these substances may account for the single athymic donor not showing cyclic AMP-raising activity in her serum.

In summary, we could find no evidence for a serum substance dependent on the presence of a thymus and acting to stimulate cyclic AMP synthesis in mouse thymocytes. We suggest that the rise in cyclic AMP in mouse thymocytes is due to a non-thymic, circulating material.

We thank Paul Schulman for technical assistance and Dr G. Goldstein for stimulating discussion.

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Received 31 March; accepted 21 November 1977.

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A. ASTALDI, G. C. B. ASTALDI, P. TH. A. SCHELLELKENS AND V. P. EIJSSVOOGEL REPLY—To date, we have investigated sera from 56 thymectomised myasthenic patients (mean age 32.1 yr, s.e.m. ± 1.8 , time post-thymectomy 1 month to 19 yr), 37 non-thymectomised myasthenic patients (mean age 44.8 yr, s.e.m. ± 3.4) and 87 normal healthy individuals (mean age 29.0 yr, s.e.m. ± 1.2). The SF activity was very low in the thymectomised donors (mean 15.6 pmol cyclic AMP per 2×10^7 cells, s.e.m. ± 3.1), but consistently demonstrable both in normal healthy individuals (mean 76.9 pmol cyclic AMP per 2×10^7 cells, s.e.m. ± 4.0) and in non-thymectomised myasthenic patients (mean 56.0 pmol cyclic AMP per 2×10^7 cells, s.e.m. ± 7.4). The difference in SF activity between normals and non-thymectomised myasthenic patients is probably due to age, because we observe that the level of SF declines progressively after the age of 30. Interestingly, within the group of the thymectomised donors two populations could be distinguished, a major one ($n = 43$) with almost no SF activity (mean 6.0 pmol cyclic AMP per 2×10^7 cells, s.e.m. ± 1.1) and a minor one ($n = 13$) with SF activity (mean 47.0 pmol cyclic AMP per 2×10^7 cells, s.e.m. ± 7.6) which is closer to that in control groups. This finding is not unexpected in view of the well known 20% incidence of ectopic thymus in humans^{1,2}; also, in some patients the thymectomy may have been incomplete. Repeated determinations of SF activity before and after thymectomy revealed that the SF activity in humans decreases dramatically after thymectomy (Fig. 1).

Sunshine *et al.* state that the cyclic AMP present in the SF should be subtracted from cyclic AMP measured in the samples after incubation. The assay method that we use, however, (see legend to Fig. 1) is rather different from that of Sunshine *et al.* Contrary to their assay, we are measuring only the net cellular cyclic AMP, for by washing the cells before the extraction of cyclic AMP, any cyclic AMP present outside the cells is removed. This could be further sustained by the finding

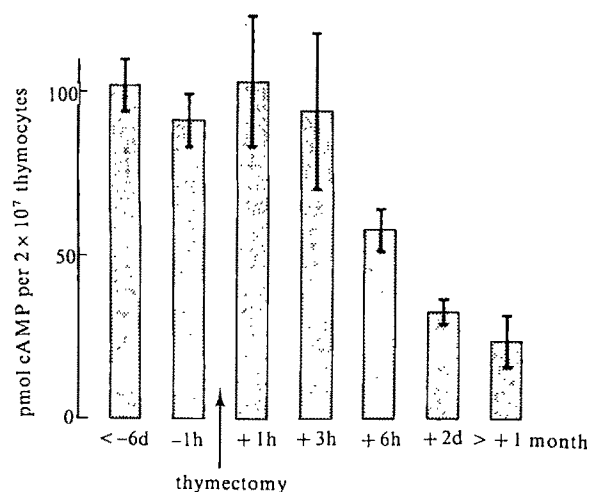


Fig. 1 SF activity at different time intervals before and after thymectomy (mean \pm s.e.m. of seven myasthenic patients). Human blood was collected and immediately defibrinated by shaking for 8–10 min with glass beads. The defibrinated blood was immediately transferred to a plastic tube kept in melting ice and centrifuged at $+4^\circ\text{C}$ for 15 min at 600g in order to obtain serum. Seven ml of serum was immediately filtered through an Amicon Centrifo CF 50 A filter (MW cut-off 50,000). Filters were centrifuged at $+4^\circ\text{C}$ for 1 h at 600g. The ultrafiltrate (SF) was divided into appropriate aliquots, frozen at -100°C and lyophilised. All samples were processed in the same way and tested within a month. We demonstrated that, once lyophilised, samples retain SF activity for a period of over 8 months. Cyclic AMP-free and prostaglandin-free SF was obtained by means of Bio-Rad AG1-X8 chromatography. SF was eluted with 0.1 N formic acid and relyophilised. As control we used SF diluted with 0.1 N formic acid and relyophilised without chromatography. Lyophilised material was reconstituted, just before assay, to the original volume of the filtrate with distilled water. Thymocytes were obtained from C57BL/6 mice aged 6–8 weeks by means of organ dissociation through a metal sieve and subsequent filtration through nylon wool columns. Each assay tube contained 2.5 ml SF, 2.5 ml Earle's solution and 10^7 thymocytes. After 5 min of incubation at 37°C , the tubes containing the cell suspension were centrifuged at $+4^\circ\text{C}$ for 5 min at 600g. Cell viability, both before and after incubation, was $>95\%$. The supernatant was discarded and the pellet resuspended in 0.5 ml of 50 mM Tris-HCl, 4 mM EDTA buffer, pH 7.5, by means of a vortex mixer. Tubes were placed in liquid nitrogen for 1 min and subsequently in a boiling water bath for 2–3 min. Coagulated proteins were spun down at $+4^\circ\text{C}$ for 5 min at 1,500g. The supernatant was directly assayed for cyclic AMP as described⁹ or further purified by means of Bio-Rad AG1-X8 chromatography. Cyclic AMP was eluted with 2N formic acid according to the method of Kuehl *et al.*³. Results were expressed in picomoles of cyclic AMP per 2×10^7 cells. Values reported here were obtained by subtracting the background cellular levels of cyclic AMP (that is, the level of cyclic AMP in thymocytes incubating as such).

that cyclic AMP-free SF (after Bio-Rad AG1-X8 chromatography) had activity similar to SF prepared without chromatography.

Sunshine *et al.* suggested that we might be measuring material other than cyclic AMP, but phosphodiesterase treatment of extracted cellular cyclic AMP confirmed that the material measured was $>90\%$ cyclic AMP. The presence of inhibitory factors is very unlikely because, when a known amount of reference cyclic AMP was added to the extracted cellular cyclic AMP, we recovered $>95\%$ of the expected value, again indicating the correctness of our cyclic AMP measurements. We have purified the extracted cellular cyclic AMP by means of ion exchange chromatography as reported by Kuehl *et al.*³ without any effect on the significance of our findings. In addition, the values of cyclic AMP that we measured in unpurified samples essentially agree with those observed by others⁴, both for isoproterenol and prostaglandin E_1 -stimulated lymphoid cells.

A further discrepancy between the results of Sunshine *et al.* and ourselves is the great difference in activity that we observed between SF prepared by rapid defibrination of fresh blood and SF obtained from blood just allowed to clot; when blood from

normal donors was allowed to clot at room temperature for 1 h, we could find almost no SF activity (mean 6.2 pmol cyclic AMP per 2×10^7 cells, s.e.m. ± 1.2 , $n = 9$). This value is similar to those reported by Sunshine *et al.*, which we regard as very low. It should be stressed that active SF preparations can only be obtained if the extraction procedure described by us is exactly followed, including rapid defibrination.

Sunshine *et al.* raised the possibility that the SF activity might be due to adrenaline or prostaglandins. This is very unlikely in view of the target cell specificity of SF as compared to the β -adrenergic stimulator isoproterenol and to prostaglandin E_1 ⁵. Hadden *et al.*⁶ demonstrated the presence of adrenergic receptors in human lymphocytes and Smith *et al.*⁷ showed that prostaglandins and β -adrenergic stimulators increase cellular cyclic AMP in human peripheral blood lymphocytes, while α -adrenergic stimulators do not. According to this view, adrenaline should stimulate cyclic AMP by means of interaction with the β -adrenergic receptors. We found⁵ that SF does not increase cyclic AMP levels in human peripheral blood lymphocytes. This rules out the possibility that the SF activity might be due to adrenaline or prostaglandins. We also reported⁵ that the β -adrenergic inhibitor propranolol does not to any extent inhibit the SF activity, indicating that SF is not acting on the β -adrenergic receptors.

We have demonstrated⁸ that the target cells for SF are among the hydrocortisone (HC)-sensitive thymocytes and that SF induced HC resistance, a maturation step reported to be induced also by a thymic extract⁹ and by a circulating thymic factor¹⁰. When mouse thymus was depleted of HC-sensitive cells by means of HC treatment *in vivo*, we found that cells recovered (HC-resistant) could not be stimulated to increase their cyclic AMP level by SF. On the contrary, prostaglandin E_1 induced HC-resistant cells to increase cyclic AMP at a level similar to that found when the total thymic population was used as prostaglandin target, again indicating that SF is not acting on the prostaglandin receptor. This could be further supported by the finding that prostaglandin-free SF (after Bio-Rad AG1-X8 chromatography) had activity similar to SF prepared without chromatography.

Finally, we found (Schellekens *et al.*, in preparation) that SF activity was almost absent in seven children with thymic-dependent immunodeficiencies and present in four children with severe combined immunodeficiency disease. Three children, almost completely lacking in SF activity, were treated with the partially purified thymic extract, thymosin fraction V, prepared by Hoffmann-La Roche according to Hooper *et al.*¹¹ Shortly after initiation of thymosin treatment, we observed¹² the appearance in the patients' blood of SF or SF-like cyclic AMP increasing activity. This activity disappeared after thymosin was stopped in one patient. Since thymosin does not induce cyclic AMP increase when tested directly on mouse thymocytes *in vitro*¹³, the SF (-like) activity found during thymosin treatment might be due to *in vivo* activation either of or by thymosin.

The only remaining possible explanation for the failure of Sunshine *et al.* to reproduce our findings seems to be the difference in technique. We realise that the description of methodology previously reported⁵ could be misinterpreted. In fact, as stated above, non-appropriate handling of the blood may cause loss of SF activity. When tested, such SF will give a very modest cyclic AMP stimulation in the range of values reported by us for thymectomised donors (ref. 5 and above), which is of exactly the same order of magnitude as that reported by Sunshine *et al.* both for normals and thymectomised individuals.

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Received 22 April; accepted 21 November 1977.

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Fibroblast-conditioned medium contains cell surface proteins required for cell attachment and spreading

PROLIFERATION in diploid fibroblasts is limited to cells that are attached and spread on a substrate. The attachment and spreading activities of normal cells are enhanced in the presence of media conditioned by the growth of diploid fibroblasts^{1,2}, and a cell surface protein (LETS/CSP)³ extracted from normal cells enhances the attachment of transformed cells^{4,5}. We report here that the activities present in conditioned medium are due to several high molecular weight glycoproteins ('released proteins'). In addition, we find that the same activities can be extracted ('extracted proteins') from the surfaces of whole cells. The results establish that growth related biological activities are transferred intercellularly through the release and absorption of cell surface components.

A comparison of released and extracted proteins is shown in Fig. 1. Figure 1a and e show that approximately 90% of the Coomassie blue staining material was located in three major bands of >150,000 apparent molecular weight (MW). The rest of the material migrated between 35,000 and 150,000 MW and was removed in the supernatant fraction after treatment with a 35% saturated solution of ammonium sulphate. The three released proteins were clearly the products of cellular synthesis. Metabolic incorporation of either ¹⁴C-leucine (Fig. 1f–h) or ¹⁴C-glucosamine (Fig. 1j) labelled all major bands. The labelling patterns of the extracted proteins (Fig. 1i and k) were similar. The band in the middle of the gels, stained with Coomassie blue (Fig. 1a, e), comigrates with bovine serum albumin and probably represents a serum contamination in those preparations. That protein is not present in the final preparations, however (Fig. 1c). In addition, the incorporation of both precursors indicated that the major bands contained both carbohydrate and protein. Colorimetric analysis of the protein mixtures by the Lowry test for protein⁶ and by a phenolsulphuric acid test for carbohydrate⁷ revealed a protein to carbohydrate mass ratio of about 10:1. These criteria suggested that the bands were composed of glycoproteins.

The similarities in the electrophoretic patterns suggested that released and extracted proteins were the same proteins and that the released proteins were components of the cell surface. That conclusion was supported by labelling whole cells with radioiodine using a lactoperoxidase system⁸ to ensure that only external components were radioactive. The electrophoretic pattern of proteins extracted from iodinated cells is shown in Fig. 1m. A duplicate culture of iodinated cells was washed to remove loosely attached peripheral components⁹ and incubated for 24 h. The iodinated released proteins (Fig. 1l) showed the same major bands as observed for extracted proteins. There were extra minor bands in the released protein fraction. The origin of those components is unknown.

Both extracted proteins and released proteins promoted the attachment and spreading of non-transformed cells. Table 1 shows the results of experiments in which low density cells were plated on glass in the absence of serum.

In these conditions very few cells attached and no cells had extended processes. The addition of bovine serum albumin 50 µg ml⁻¹ had no effect. However, the addition of 50 µg ml⁻¹ of extracted or released proteins resulted in > 90% of the cells spread within 20 h. The partial fractionation of the released and extracted proteins by ammonium sulphate precipitation showed that the activity was present in material that precipitates in a solution that is less than 35% saturated and that most of the activity is in the 20–35% saturated fraction (Table 1). Although this fractionation procedure is imperfect, it is reasonable to conclude that the spreading and attachment activities were associated with the presence of high molecular weight species. We were unable to detect any low molecular weight

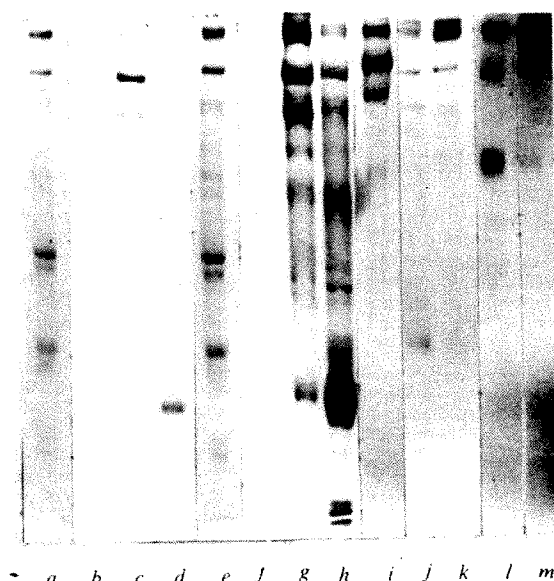


Fig. 1 Comparison of released and extracted proteins. All experiments used a single primary culture of human diploid fibroblasts that was derived from a foreskin biopsy and initiated in this laboratory. The cells were cultured in medium 199 (GIBCO) supplemented with 20% foetal bovine serum (Flow) and antibiotics. Released proteins were obtained as described previously¹. Extracted proteins were obtained by the method developed for chick cell surface proteins¹⁵ using 1 M urea. To obtain ¹⁴C-labelled proteins, confluent monolayers were pulsed for 24 h with 0.3–0.5 µCi ml⁻¹ ¹⁴C-amino acids (280 mCi mmol⁻¹) or ¹⁴C-glucosamine (60 mCi mmol⁻¹) (New England Nuclear). The monolayers were then washed to remove all traces of serum and the medium was replaced with serum-free medium 199 containing 0.3–0.5 µCi ml⁻¹ of the same labelled compound. After 24 h the released proteins were gently decanted and the cells were washed. Proteins were then extracted from the washed cells. ¹²⁵I-labelled released proteins were obtained by labelling a washed monolayer for 2 min with 500 µCi ml⁻¹ sodium iodide (NEN) using a lactoperoxidase-catalysed procedure⁸. The labelled cells were then incubated for 7–20 h, in serum-free medium 199. Electrophoretic separation of dithiothreitol-reduced samples was obtained by standard procedures¹⁶. Samples were heat denatured in 0.1% sodium dodecyl sulphate (SDS) and electrophoresed through a 5% polyacrylamide stacking gel (not shown) and a 7.5% polyacrylamide separating gel. This figure is a composite of several experiments performed at different times. Autoradiograms of the gels (tracks g–m) were obtained by exposing prefogged Kodak X-ray film to dried gels for 3–21 d at –60 °C. Protein concentrations in the 13 tracks range from 40 to 250 µg. Tracks a–e: Coomassie blue stained: a, released protein; b, 20% (NH₄)₂SO₄ released protein; c, 35% (NH₄)₂SO₄ released protein; d, 70% (NH₄)₂SO₄ released protein, and e, extracted protein. Tracks f–i: ¹⁴C-amino acid labelled: f, 20% (NH₄)₂SO₄ released protein; g, released protein; h, 70% (NH₄)₂SO₄ released protein, and i, 35% (NH₄)₂SO₄ extracted protein. Tracks j, k: ¹⁴C-glucosamine labelled: j, released protein and k, extracted protein. Tracks l and m: ¹²⁵I-labelled: l, released protein and m, extracted proteins. The three major bands near the top of the gels (shown most clearly in (c)) have apparent molecular weights > 150,000. The abbreviation AMS indicates that the protein was insoluble in the stated concentration of ammonium sulphate.

Table 1 Spreading activity of released and extracted proteins

Sample	No. of cells		% Spread
	Attached	Spread	
Control	7	0	0
+ BSA	6	0	0
Released proteins	2	98	98
20% (NH ₄) ₂ SO ₄	28	27	49
35% (NH ₄) ₂ SO ₄	34	102	75
70% (NH ₄) ₂ SO ₄	9	1	10
Extracted proteins	4	98	96
35% (NH ₄) ₂ SO ₄	7	96	93

All numbers represent the means of at least two independent determinations. The control is medium 199 without protein. All other samples contain 35–45 µg of protein in medium 199. Released and extracted proteins were precipitated sequentially with 20%, 35%, and 70% saturated ammonium sulphate solutions at 0 °C. Attached and spread cells are determined by phase contrast microscopy according to the parameters suggested by Taylor¹⁷.

species on either 7.5% or 20% polyacrylamide gels (data not shown) even at concentrations as high as 5 mg ml⁻¹ (50 µg ml⁻¹ is required for > 95% spreading). Electrophoretic profiles of ammonium sulphate-precipitated materials (Fig. 1 *b–d, f–h*) indicated that the 20–35% fraction contained the three high molecular weight species.

The data may be interpreted as indicating either that the spreading activity and the attachment activity were due to separate components or that one protein promotes both activities at high concentration and only attachment at low concentrations. Table 2 shows that over 50% of the spreading activity was absorbed to fixed cells, but that cell attachment was unaffected by absorption. Trypsin-treated cells were washed and fixed before absorption to prevent the production and release of the proteins under investigation. The spreading activity was substantially reduced in the absorbed solution, but the total number of attached plus spread cells remained about the same for absorbed material as it was for non-absorbed material. Other experiments using radioactive released proteins showed that in the conditions of these experiments the proteins absorbed to the cells and not to the plastic container (data not shown). The data presented here show that cell surface components are active in cell attachment and spreading and that those components are released, in an active form, into the culture media. The function of that turnover is not known, however. We propose that the release and absorption of cell-surface components is a form of intercellular communication that promotes cell growth. Previously, we demonstrated

that cells plated at high density attached and spread in the absence of serum while low density cells required non-dialysable components present in conditioned medium¹. We suggest that at high density the concentration of cell surface-derived released proteins is high enough to maintain a critical concentration of those proteins on the cell surface. However, at low density, the equilibrium between the concentration of released proteins on the cell surface and in the culture medium is shifted so that the surface concentration is too low to support attachment and spreading. It is possible that the low-density cells have an altered physiology¹⁰, but our experiments do not test this.

We do not know which of the released proteins are responsible for the activities tested. However, the involvement of cell-surface components in cell adhesion is well established. A LETS/CSP protein has been shown to be active in red cell agglutination¹¹ and in the adhesion of transformed cells^{1,5}. In addition, there is considerable evidence to indicate that cell-membrane components are released into the culture media^{12,13} and that a serum component is antigenically identical to LETS/CSP¹⁴. It is possible that the large band at the top of the gel is identical to LETS/CSP³ and is responsible for some of the activity. However, it is also possible that more than one protein is involved since attachment and spreading activities seem to be due to separate components (Table 2).

This work was supported by grants from the US NIH (NICHD, NIA). We thank Bob Speck for photographic assistance. A.J.T.M. is also affiliated with the Department of Pediatrics of the Albany Medical College, Albany, New York.

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Table 2 Absorption of released protein spreading activity

Sample	Attached + spread = total			% Spread
Control	8	0	8	0
Released protein (50 µg ml ⁻¹)	3	100	103	97
Absorbed solution	60	49	109	45
Released protein (25 µg ml ⁻¹)	31	58	89	65
Absorbed solution	80	27	107	25

Values represent the means of two determinations per sample. Absorption procedure: released proteins were absorbed to whole fixed cells by incubating a cell suspension (1.8 × 10⁷ cells per ml of 50 µg released protein) for 10 min at 37 °C. After the incubation the mixture was centrifuged to pellet the cells and the supernatant fraction retained as the 'absorbed solution'. The cells used for absorption had been treated with 50 µg ml⁻¹ trypsin solution for 10 min at 37 °C and subsequently fixed in 3% formaldehyde. Fixed cells were washed extensively and used without further delay. Residual proteolytic activity was not detected.

Calcium effects on gap junction structure and cell coupling

PROTOPLASMIC molecules of low molecular weight are known to travel freely across the boundaries between most neighbouring cells through small intercellular channels generally thought to be at the intramembranous particles of gap junctions^{1–3}. The permeability of the channels can be decreased to a complete interruption of cell communication by a variety of treatments^{2–10}. Most of these treatments raise the concentration of ionised calcium, [Ca²⁺], in the cytoplasm⁶, suggesting that calcium may be the uncoupling agent in both vertebrate⁹ and invertebrate cells^{2,8}. In some systems glutaraldehyde fixation has also been shown to produce uncoupling³; however, this type of uncoupling does not seem to be triggered by calcium¹⁰. In parallel with functional uncoupling reversible structural changes have been described in gap junctions of crayfish characterised by an increase in tightness and regularity of particle aggregation

and a decrease in junctional thickness and particle size¹⁰. These changes, interpreted as reflecting conformational rearrangements in the protein framework of the channels resulting in channel obliteration, were recently confirmed in the rat¹¹ although in the latter, functional uncoupling was not measured electrophysiologically, but only presumed on the basis of reasonable comparative arguments. It remains uncertain whether or not the changes in both the gap junction structure and the cell coupling are due to a direct action of calcium on the junctional membranes. We show here that the junctional change is indeed a calcium effect, triggered by $[Ca^{2+}]$ as low as 5×10^{-7} M.

Gap junctions of the eye lens of calf were studied by freeze-fracture either in intact cells or in isolated preparations. The lenses were dissected from the eyes 30–60 min after death. For experiments on intact cells the lenses were fixed in glutaraldehyde, infiltrated with glycerol (as cryoprotective agent) and processed for freeze-fracture as described elsewhere¹¹. In these preparations, gap junctions between epithelial cells of the anterior lens surface showed particles and complementary pits organised in regular hexagonal arrays with unit cells of ~ 8.5 nm (Fig. 1a), the crystallinity of the array being more pronounced in the pitted face, as described in other gap junctions¹², while gap junctions between fibre cells displayed particles and pits irregularly packed at various spacings (Fig. 1b). This difference, reported also by Benedetti *et al.*¹³ is probably a consequence of the difference in metabolic properties between epithelial and fibre cells. The tightly and regularly packed epithelial junctions are identical to rat liver and stomach junctions uncoupled by inhibitors of the metabolism and are likely to be in an uncoupled state as well, since the lens epithelium, known to possess an active oxidative metabolism¹⁴, is expected to suffer the effects of severe hypoxia if it is not fixed immediately after animal death. In fact the inhibition of the metabolism and the consequential depletion of energy stores caused by hypoxia¹⁵ are known to raise the cytoplasmic $[Ca^{2+}]$ and result in cell uncoupling⁸ as Ca^{2+} diffuses in the cytosol from the mitochondria and the surface membrane¹⁶. Fibre cells, on the other hand, derive their energy requirement from glycolytic metabolism¹⁴ and therefore are rather insensitive to hypoxia; this is probably the reason that their gap junctions display structural characteristics of normally coupled junctions.

For studies on isolated fibre cell junctions, eight to ten lenses per experiment were homogenised either in sodium bicarbonate buffer (1×10^{-3} M, $pH = 7.4$) or in the same buffer containing EDTA 1×10^{-2} M to chelate divalent cations. The homogenate was washed four times in bicarbonate or bicarbonate-EDTA buffer respectively, centrifuged to a pellet and freeze-fractured after glutaraldehyde fixation and cryoprotective treatment. Due to the great number of gap junctions between fibre cells, the

Fig. 1 Freeze-fracture replicas of gap junctions in intact cells of calf lens. *a*, Gap junction between epithelial cells of the anterior lens surface. Particles and complementary pits form a regularly hexagonal array with a unit cell of ~ 8.5 nm. *b*, Gap junction between fibre cells. Particles and pits are randomly distributed at variable spacings. P, face P (protoplasmic membrane leaflet); E, face E (exoplasmic membrane leaflet). ($\times 160,000$).

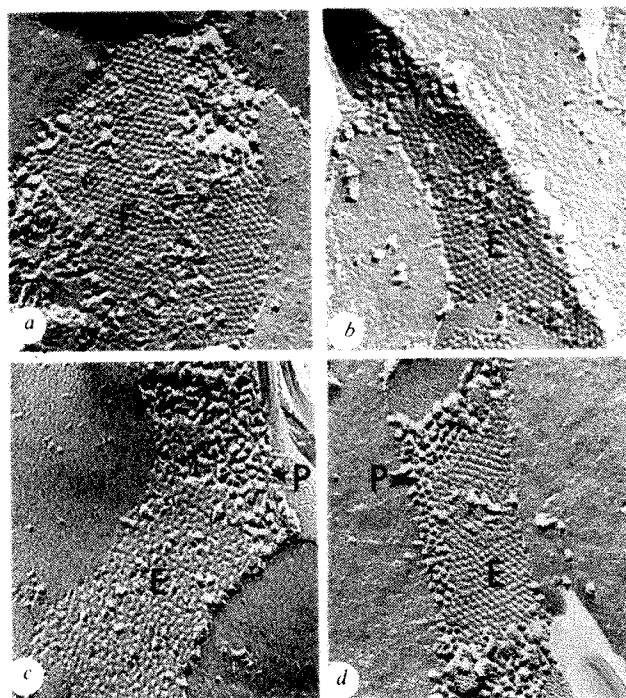
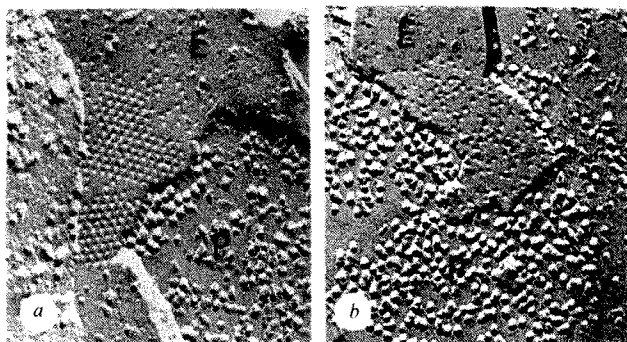


Fig. 2 Freeze-fracture replicas of gap junctions isolated from fibre cells of calf lens. *a*, Junction isolated in absence of EDTA. *b*, Junction isolated in absence of EDTA and subsequently incubated in 3×10^{-3} M EGTA. *c*, Junction isolated in presence of 1×10^{-2} M EDTA. *d*, Junction isolated in presence of 1×10^{-2} M EDTA and subsequently incubated in a 1×10^{-3} M calcium solution. Notice that junction isolation in absence of chelators of divalent cations causes aggregation of junctional particles into tight hexagonal arrays with unit cells of 7–8 nm (*a*). The phenomenon is not reversed by EGTA (*b*) or EDTA treatment, but can be prevented if the junctions are isolated in presence of EDTA (*c*). Exposure of junctions isolated in EDTA to calcium solutions causes hexagonal packing of the junctional particles (*d*). P, face P; E, face E. ($\times 160,000$).

crude pellets obtained are sufficiently rich in junctions not to require detergent treatment, and thus offer an excellent source of relatively undamaged gap junctions. Junctions isolated without EDTA display particles and pits tightly and regularly packed into hexagonal arrays with unit cells of 7–8 nm (Fig. 2a) while junctions isolated in bicarbonate-EDTA buffers have particles and pits loosely and irregularly aggregated (Fig. 2c). If junctions isolated in the presence of EDTA are suspended for 30 min at $37^\circ C$ in calcium solutions (1×10^{-5} – 3×10^{-3} M), buffered to $pH 7.4$ with 1×10^{-3} M Tris, particles and pits aggregate tightly into regular hexagonal arrays (Fig. 2d). Control junctions, suspended for the same length of time and at the same temperature in 1×10^{-2} M EDTA solutions ($pH = 7.4$) maintain the irregularly and loosely packed particle arrays (same as Fig. 2c). Junctions treated with calcium solutions do not recover the control appearance if they are subsequently exposed to 1×10^{-2} M solutions of EDTA or 3×10^{-3} M EGTA (Fig. 2b).

In junctions with regular hexagonal arrays the particles are ~ 1 nm smaller than in the others and appear more homogeneous in size and shape. In addition, in these junctions the fracture plane tends to remain within the same membrane, while in the irregularly packed junctions it steps frequently up and down from one to the other membrane. In most junctions there are a few particles which fracture away with the external leaflet, appearing as bumps on face E (Figs 2, 3). Particles on the pitted face of gap junctions are uncommon in vertebrates but are frequent in crayfish¹⁷; in the rat they are a common feature of gap junctions of livers perfused with hypertonic sucrose¹¹.

The finding of hexagonal particle packings in fibre cell junctions isolated in the absence of EDTA (Fig. 2a) suggested that trace amounts of divalent cations may be sufficient to cause the particle-clumping phenomenon. Therefore experiments using

EDTA-calcium buffers were performed in an attempt to define the triggering $[Ca^{2+}]$. Junctions isolated in bicarbonate-EDTA solutions, as previously described, were incubated for 30–120 min at 37 °C in calcium-EDTA solutions in which $[Ca^{2+}]$ was buffered to different levels ranging from $1 \times 10^{-8} M$ to $1 \times 10^{-6} M$. Calcium-EDTA solutions were prepared by dissolving calculated amounts of $CaCl_2$ into $1 \times 10^{-2} M$ solutions of EDTA buffered to pH 7 with $2 \times 10^{-2} M$ HEPES (Sigma). The amounts of $CaCl_2$ to be added were calculated using an EDTA dissociation constant for calcium of $5 \times 10^{-8} M$ (at pH 7, $\log K = 7.3$) (ref. 18). The following equation was used:

$$[Ca^{2+}]_f = ([Ca^{2+}]_t[EDTA]_t + [Ca^{2+}]_f^2 + [Ca^{2+}]_f K_D) / (K_D + [Ca^{2+}]_f)$$

where $[Ca^{2+}]_t$ represents the amount of total calcium per unit volume of medium, which gives a concentration of free calcium, $[Ca^{2+}]_f$, when the total amount of EDTA per unit volume of medium is $[EDTA]_t$, and K_D is the dissociation constant of the reaction: $Ca + EDTA \rightleftharpoons Ca-EDTA$. In each experiment the junctions were washed, before incubation, in the same calcium-EDTA solution to be used for incubation, so that residual amounts of EDTA left in the same sample after the preliminary isolation procedure would not interfere with the EDTA-calcium buffers. To make certain that changes in pH did not take place during incubation, the pH of the solutions was measured both at the beginning and at the end of the incubation period. No effect was detected on the structure of the junctions with solutions in which the $[Ca^{2+}]$ was buffered to values ranging from $1 \times 10^{-8} M$ to $3 \times 10^{-7} M$ (Fig. 3a, b), while solutions of $5 \times 10^{-7} M$ (Fig. 3c) or greater (Fig. 3d) $[Ca^{2+}]$ consistently caused the junctional particles and pits to aggregate tightly into regular hexagonal arrays. 89.3% of all the junctions exposed to $[Ca^{2+}]$ greater than $5 \times 10^{-7} M$ showed hexagonal particle packing (13 experiments were performed and 131 junctions were photographed), and 99% of all the junctions exposed to $[Ca^{2+}]$ lower than $5 \times 10^{-7} M$ showed

irregular particle packing (11 experiments were performed and 114 junctions were photographed). Interestingly, the threshold of intracellular $[Ca^{2+}]$ for functional uncoupling is believed to be somewhere between $1 \times 10^{-7} M$ and $5 \times 10^{-8} M$ in salivary gland cells of insect larvae^{19,20} and slightly larger than $2 \times 10^{-7} M$ in cow and calf heart muscle²¹ (because of the close relationship between the onset of uncoupling and the onset of muscle tension) which fits reasonably well with $5 \times 10^{-7} M$ $[Ca^{2+}]$ obtained for the changes in gap junction structure.

In conclusion, the exposure of isolated gap junctions to $5 \times 10^{-7} M$ or greater $[Ca^{2+}]$ affects the junctional structure similarly to uncoupling treatments performed on intact cells. Since uncoupling is believed to be triggered by an increase in $[Ca^{2+}]$ in the cytoplasm, the calcium dependent changes in gap junction structure described here confirm the hypothesis^{10,11} that gap junctions with tightly packed hexagonal arrays of particles correspond to non-permeable junctions and indicate that calcium affects both the structure of the junctions and the cell coupling by acting directly on the junctional molecules. These data also suggest that small fluctuations from the normal intracellular $[Ca^{2+}]$, believed to be $1 \times 10^{-7} M$ (ref. 16), could be sufficient to produce functional uncoupling. Our findings do not, however, necessarily indicate that calcium is indeed the only agent capable of triggering the junctional changes with uncoupling. A recent report²² suggesting the uncoupling effects of low pH poses the question as to whether or not the structure of the junctions can also be affected in a similar way by acidic free calcium solutions. Experiments using such solutions are underway. Evidence from ultrastructure^{10,11} and X-ray diffraction^{23,24} suggests that the structural change is not a mere modification in particle packing but rather a conformational rearrangement in the molecular framework of the channels. The mechanism that causes the rearrangements in particle array is not known; however, it is interesting that it is triggered by a $[Ca^{2+}]$ similar to that²⁵ which causes muscle contraction. Although the presence of an actomyosin-like system associated with gap junction membranes has never been suggested, there are no reasons for discarding it *a priori*.

I thank Mrs Lillian Peracchia for technical assistance. This research was supported by a grant from the USNIH (1 RO1 GM 20113).

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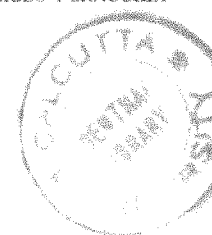
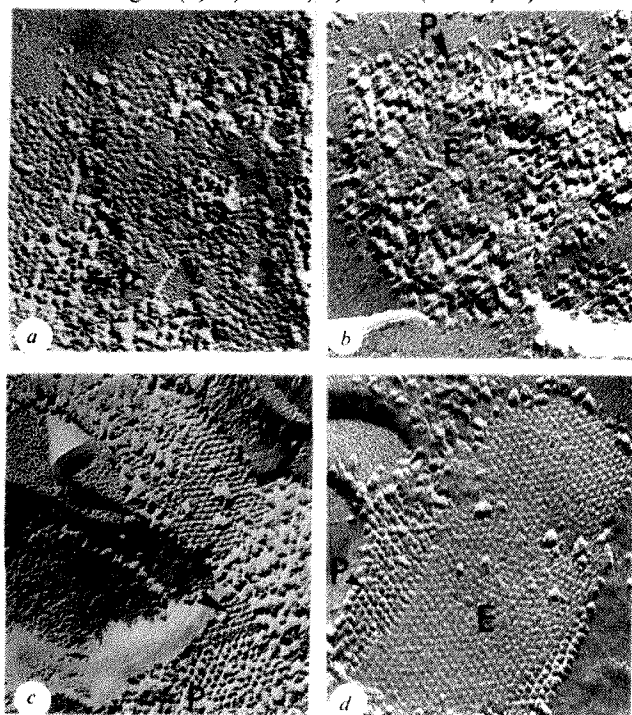


Fig. 3 Freeze-fracture replicas of gap junctions isolated from fibre cells of calf lens. All the junctions have been isolated in presence of $1 \times 10^{-2} M$ EDTA and subsequently washed and incubated in calcium-EDTA solutions (pH 7.0) in which $[Ca^{2+}]$ was buffered to $1 \times 10^{-7} M$ (a), $3 \times 10^{-7} M$ (b), $5 \times 10^{-7} M$ (c) and $1 \times 10^{-6} M$ (d). Note that particles and pits aggregate tightly into regularly hexagonal arrays only at a $[Ca^{2+}]$ of $5 \times 10^{-7} M$ (c) or higher (d). P, face P; E, face E. ($\times 160,000$).



Received 16 July; accepted 28 November, 1977.

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Effects of fusogenic agent on membrane structure of erythrocyte ghosts and the mechanism of membrane fusion

MEMBRANE fusion clearly requires that participating lipids assume some transitory non-bilayer configuration during the intermediate stages. Previous workers have suggested that intermediate micellar¹ or inverted micellar^{2,3} structures may occur, but the precise nature of possible intermediates and their relation to the physical properties of membrane lipids are obscure. In this regard, Lucy and co-workers⁴⁻⁸ have shown that 'fusogenic' agents such as fatty acids and their derivatives induce erythrocytes to fuse. Such agents might possibly promote fusion by enabling endogenous lipids to assume non-bilayer configurations. We have therefore investigated the influence of two such fusogens on the structure of the erythrocyte (ghost) membrane using ³¹P NMR techniques, which have been found to be sensitive to phospholipids in non-bilayer phases^{9,10}. We show that the incorporation of oleic acid and glycerol mono-oleate into the ghost membrane, at concentrations similar to those needed to induce cell fusion between erythrocytes *in vitro*, produce a well-defined transition of a variable portion of the membrane phospholipids from the bilayer phase to an hexagonal (H_{II}) phase. These results lead us to propose a model for membrane fusion induced by oleic acid, which we suggest may also apply to fusion events *in vivo*.

The phases available to hydrated liquid crystalline phospholipids, and the corresponding ³¹P NMR spectra obtained are illustrated in Fig. 1. Briefly, the phospholipids in (large) bilayer structures give rise to broad asymmetric ³¹P NMR spectra with a low field shoulder⁹⁻¹⁴. Alternatively, phospholipids in the hexagonal (H_{II}) phase (ref. 15) exhibit narrower

spectra with a high field shoulder¹⁰. Finally, phospholipids in other available phases exhibit much narrower symmetrical ³¹P NMR spectra¹⁰.

The 36.4 MHz ³¹P NMR spectra obtained from erythrocyte ghosts incubated in the presence of increasing amounts of oleic acid are shown in Fig. 2. A progressive conversion of the membrane phospholipids from the bilayer phase to the hexagonal (H_{II}) phase is observed. Similar effects were also observed for glycerol mono-oleate. Such spectra cannot arise from lipids in

Fig. 1 Phospholipid phases and corresponding (36.4 MHz) ³¹P NMR spectra observed. The 36.4 MHz ³¹P NMR spectra were obtained from (unsonicated) aqueous dispersions of egg yolk lecithin at 30 °C (bilayer phase), soya phosphatidylethanolamine at 30 °C (hexagonal H_{II} phase) and a mixture of soya phosphatidylethanolamine and 15 mol% egg yolk lecithin at 30 °C (cubic, rhombic or inverted micellar phase, for fuller details see ref. 10). All aqueous dispersions contained 25 mM Tris-HAc (pH 7.1) and 2 mM EDTA. All spectra were obtained in the presence of high power (18 W) broad band proton decoupling.

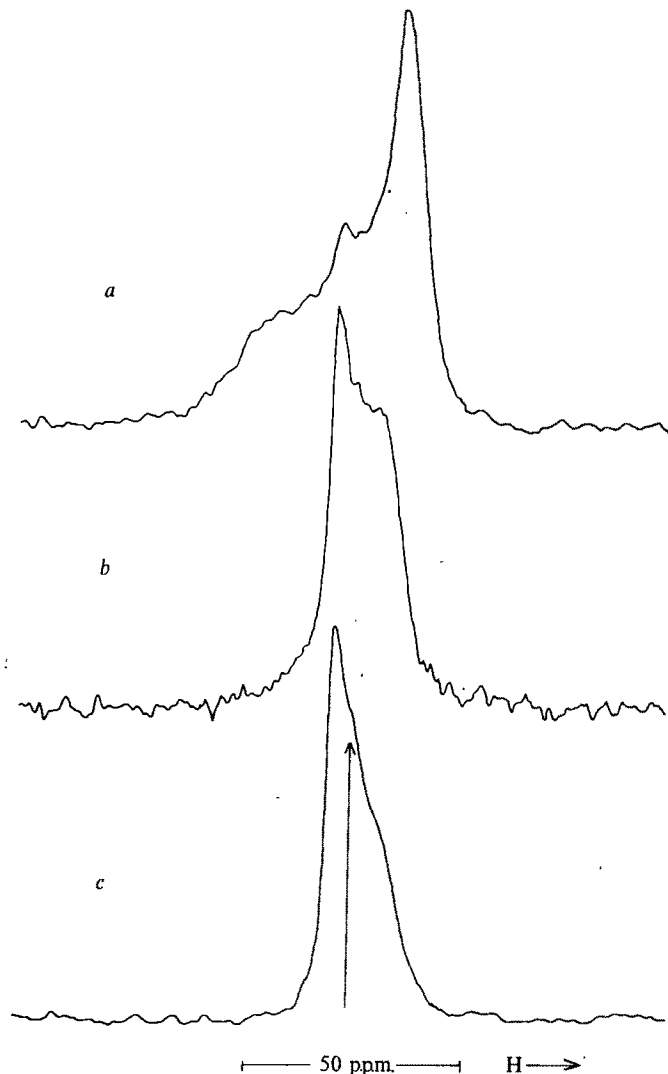
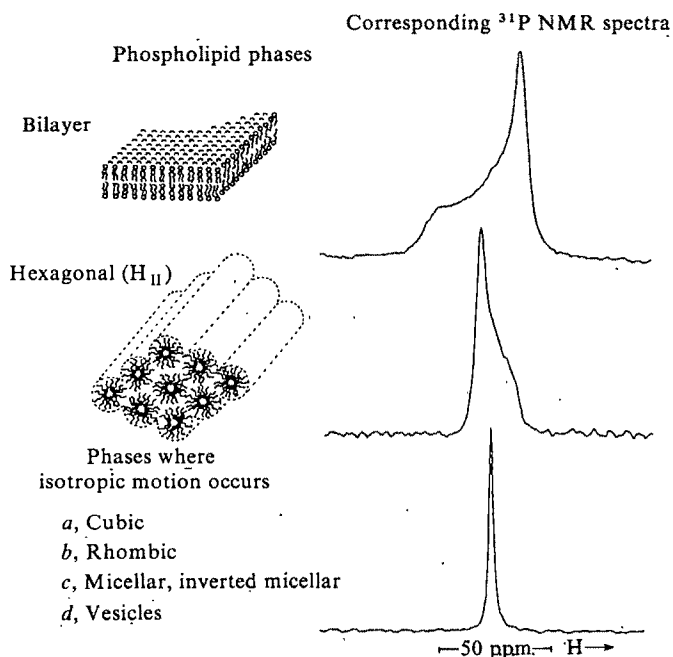


Fig. 2 36.4 MHz ³¹P NMR spectra obtained at 37 °C from a, 150 mg (dry weight) erythrocyte ghost incubated in medium A (for details of incubation conditions and composition of medium A, see below) in the absence of oleic acid; b, 150 mg (dry weight) erythrocyte ghost incubated in medium A containing 35 mg oleic acid; c, 150 mg (dry weight) erythrocyte ghost incubated in medium A containing 50 mg oleic acid. The arrow indicates the position of ³¹P NMR spectra arising from sonicated vesicles. Erythrocyte ghosts were prepared from human bank blood as detailed previously²⁴ and were subsequently lyophilised and stored under N₂ at -20 °C before use. Medium A contained NaCl (0.12 M), KCl (6 mM), MgSO₄·7H₂O (5 mM), CaCl₂·2H₂O (2 mM) and Tricine (20 mM), and the pH adjusted to 6.8. The oleic acid was added drop by drop to 40 ml of medium A and subsequently sonicated (5 min) to obtain a milky suspension which was added to the (dry) ghost material. The resulting mixture was incubated at 37 °C for 20 min while stirring continuously. The material was subsequently centrifuged (50,000g) for 10 min at 0 °C and 0.1 ml D₂O (25 mM Tris-HAc, pH 7.0) added to the pellet, which was then transferred to the NMR tube. Spectra were obtained from up to 40,000 transients in the presence of high power (20 W) proton decoupling, using a 45° resonance frequency pulse and a 0.18 s interpulse time. Fatty acid determinations (see text) indicated an oleic acid to phospholipid ratio (mol/mol) of 1.3 for sample (b) and 2.4 for sample (c).

micellar or inverted micellar phases, or from bilayer phase lipids in rapid exchange with such micellar phases, as either situation would give rise to a narrow spectral component (≈ 20 Hz linewidth) with a chemical shift characteristic of isotropic averaging. Freeze fracture electron micrographs obtained from the sample of Fig. 2c reveal (Fig. 3) that the protein is segregated away from regions of the H_{II} phase, which exhibit a characteristic striated pattern consistent with previous freeze fracture visualisations of the H_{II} phase in model systems¹⁶.

The formation of the H_{II} phase was observed to be strongly dependent on the presence of Ca^{2+} in the incubation medium. Incubations equivalent to that performed for the sample of Fig. 2c but where Ca^{2+} was omitted resulted in broad, rather featureless ^{31}P NMR spectra which could not be clearly identified with either the bilayer or H_{II} phases. Control experiments (see below) showed that similar concentrations of oleic acid were associated with the ghost membrane in both situations. ^{31}P NMR studies were also performed on liposomes composed of the total lipids extracted from the erythrocyte membrane (to which various amounts of fusogen had been added prior to hydration) in the presence and absence of Ca^{2+} . Addition of Ca^{2+} to these systems promoted the formation of the H_{II} phase (above a fusogen/phospholipid ratio of 1 mol/mol). This provides strong evidence that Ca^{2+} directly facilitates the formation of the H_{II} phase in the ghost membrane. This apparent ability of Ca^{2+} to induce bilayer to hexagonal (H_{II}) phase transitions may be related to the previously noted^{17,18} ability of Ca^{2+} to cause lateral phase separation of charged lipid species in mixed lipid systems.

If the H_{II} phase formed in the fusogen-treated erythrocyte ghost membranes is relevant to the mechanism by which fusogens facilitate fusion between erythrocytes *in vitro*, similar amounts of fusogen should be associated with ghosts and erythrocytes when significant hexagonal (H_{II}) phase or fusion, respectively, is observed. Thus fusion was induced between erythrocytes, employing oleic acid, in the manner detailed previously⁷. The cells were centrifuged on observation of initial fusion events (10–20% fusion, as observed in a phase contrast microscope), and a fatty acid analysis of the pellet allowed a determination of the oleic acid associated with the cells. Excess fatty acid floated on the supernatant after centrifugation. Approximately 1 mol of oleic acid per mol of erythrocyte phospholipid was required before significant fusion occurred. This may be compared to an oleic acid/phospholipid ratio of 1.3 (mol/mol) obtained for the sample of Fig. 2b where significant formation of the H_{II} phase is observed. Assuming that a similar proportion of the membrane associated fusogen is contained in the ghost and erythrocyte bilayers respectively, these results indicate that membrane concentrations of fusogen sufficient to initiate fusion in erythrocytes are also sufficient to promote formation of the H_{II} phase in the ghost membrane. This suggests that observation of the H_{II} phase in the ghost system corresponds to ghost fusion (although this has not yet been observed by other techniques) and also suggests that formation, or a tendency to formation, of the H_{II} phase in the erythrocyte membrane itself is vital to subsequent fusion events.

On the basis of this information we propose a model of cell fusion induced by oleic acid which is illustrated in Fig. 4. The fusion event is envisaged to proceed through (at least) four stages; these involve aggregation of protein to produce areas of (protein free) lipid bilayer¹⁹, which subsequently make contact (Fig. 4b). It is then suggested that the two outer monolayers combine to form an intermediate hexagonal H_{II} phase (Fig. 4c) which is subsequently restabilised to bilayer structure (Fig. 4d) to complete the fusion process. The presence of Ca^{2+} is probably important at each stage, indeed Ca^{2+} has already been implicated in the processes of protein aggregation and cell-cell contact²⁰. We emphasise, however, that the presence of Ca^{2+} would seem to be particularly important for the formation of the H_{II} phase of Fig. 4c. Further, the resta-



Fig. 3 Freeze-etch electron micrograph obtained from erythrocyte ghost sample giving rise to the spectra of Fig. 2c. The sample was quenched from 20 °C. Other conditions were as detailed previously²⁵. Magnification $\times 250,000$.

bilisation event may be envisaged to occur by way of an active (ATP-dependent) removal of Ca^{2+} from the cytoplasm of the partially fused cells of Fig. 4c, thus producing a strong chemical potential between the aqueous pores of the H_{II} phase and the cytoplasm. This would encourage the removal of Ca^{2+} and subsequent reversion of the H_{II} lipid to the bilayer phase.

There are strong indications that the mechanism of membrane fusion shown in Fig. 4 may also apply to naturally occurring membrane fusion *in vivo*. In particular, many species of naturally occurring lipids found in significant concentrations in biological membranes prefer the hexagonal (H_{II}) phase or other inverted phases (intermediate formation of inverted micelles would be equally acceptable to the fusion model of Fig. 4) over bilayer structure. Notable examples include unsaturated phosphatidylethanolamines^{9,10,21,22} and cardiolipin²³. Moreover, the formation of H_{II} phases for both these lipid species may be facilitated by the presence of Ca^{2+} (ref. 23 and P. R. Cullis, unpublished). Cholesterol also tends to favour the formation of hexagonal (H_{II}) phases in certain situations¹⁰. Thus 'natural fusogens' abound, and segregation of such non-bilayer lipids into particular regions of the membrane (in the presence of Ca^{2+}) would be expected to facilitate fusion events. Cell-cell contact in the form of Fig. 4c may also correspond to 'quasi-fusion' events²⁰ associated with tight junctions.

We thank Professor L. L. M. van Deenen for his support and for laboratory facilities and Dr A. J. Verkleij for performing

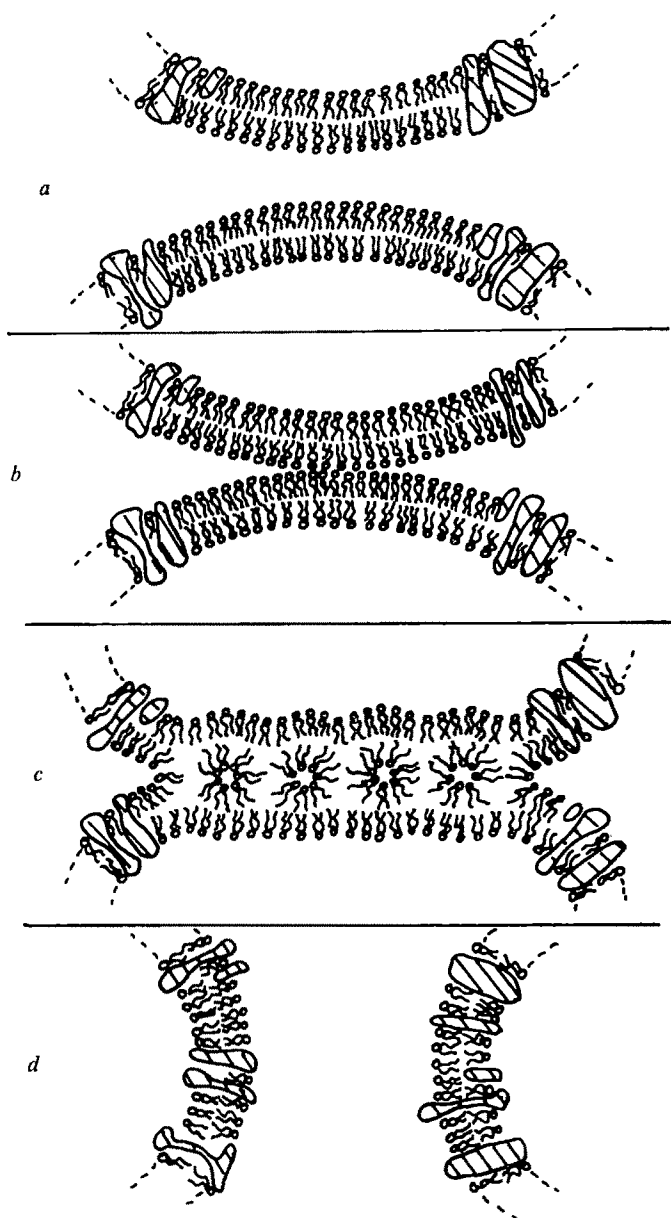


Fig. 4 Proposed mechanism of membrane fusion. The slashed areas traversing the membrane indicate integral membrane protein. Note that the diameter of the aqueous channels of the hexagonal phase component of part c are drawn approximately to scale with respect to the thickness of the bilayer.

the freeze-etch study. P. R. C. acknowledges financial support from EMBO, and M. J. H. thanks the Netherlands Ministry of Education and Science and EMBO for support.

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Distribution of sialic acids on the red blood cell membrane in β thalassaemia

SIALIC acids (SA) of the red blood cell (RBC) membrane are considered to play an important part in the physiology of the RBC¹. There are indications that the amount of membrane sialic acid is a major factor in distinguishing young from aged RBC. Thus, ageing of circulating RBC is associated with a reduction of 20–30% SA which may be an important determinant of RBC survival^{2,3} in the eventual recognition and sequestration of the latter cells by the reticuloendothelial system². During studies of thalassaemic RBC we observed, among other alterations, that the average level of SA was approximately 25% less than in normal RBC membranes obtained from healthy donors⁴. We, therefore, investigated the comparative ultrastructural distribution of SA on the surface of thalassaemic RBC membranes. We found that the SA residues on the surface of thalassaemic RBC are distributed in an uneven manner and are less abundant than those present on normal RBC surfaces.

We used three cytochemical methods in conjunction with transmission electron microscopy, in order to detect SA on membranes of intact RBC. One method is based on the production of a biotinyl derivative of SA which can be visualised by ferritin-avidin conjugates as described previously^{5,6}. In addition, two other techniques were used, cationised ferritin (CF) (ref. 7) and ruthenium red⁸, both of which interact with negatively charged sites on the membrane. The latter techniques were used because membrane-based sialyl residues are known to contribute most of the negative charge on the surface of the human RBC⁹.

The results obtained from all three techniques are illustrated in Fig. 1. The distribution of the above membrane probes from representative thalassaemic RBC samples was compared with that of normal RBC. In the former cases, only cells with characteristic thalassaemic morphology^{10,11} were evaluated. Both normal and thalassaemic RBC stained with ruthenium red, and a continuous layer of electron-dense material was observed. The deposit was much less dense, however, on the surface of thalassaemic RBC (Fig. 1a) than on normal cell membranes (Fig. 1b).

Unlike the ruthenium red stain, which results in an amorphous layer of reaction products, ferritin-based stains are characterised by a particulate marker of uniform size. The ferritin label can be quantitatively evaluated by enumeration and enables a finer resolution of the given surface site. Thus, quantification of cationised ferritin (CF) on micrographs of tangentially sectioned membrane (Figs 1c and d) revealed less attached CF particles per μ^2 on thalassaemic cells ($1,200 \pm 275 \sigma$) than on normal RBC ($2,030 \pm 150 \sigma$). In some instances the CF label on the former cell membranes was interrupted by unlabelled gaps (Fig. 1c, arrows), in contrast to the more even distribution of the label on normal RBC.

Additional details of the distribution of membrane SA were obtained by a more specific technique using ferritin-

avidin conjugates to visualise biotinylated sialyl residues. Examination of perpendicularly sectioned membranes obtained by this technique reveals the topographical resolution of sialyl residues from the lipid bilayer^{6,7}. With the above method, ferritin particles on the surface membrane of thalassaemic RBC are distributed in patches separated by unlabelled gaps (Fig. 1e). In addition, a distance of up to 300 Å could be discerned between some of the aggregated ferritin particles and the plane of the membrane surface. These observations may indicate either that some SA residues on thalassaemic RBC are associated with oligosaccharides of a more complex nature or that these sites have been displaced from the membrane surface. These phenomena were absent in normal RBC labelled in the same manner. In the normal RBC membrane, ferritin particles were almost uniformly distributed and protruded about 50–100 Å from the outer electron-dense layer of the cell membrane (Fig. 1f).

These ultrastructural observations support biochemical data which indicate that less SA are present on the surface membrane of thalassaemic RBC⁴. Similar observations on RBC membranes obtained from patients with β -thalassaemia intermedia are summarised in Table 1. Although the latter form of thalassaemia is morphologically indistinguishable from β -thalassaemia major, the clinical course is less severe, and the patients do not require

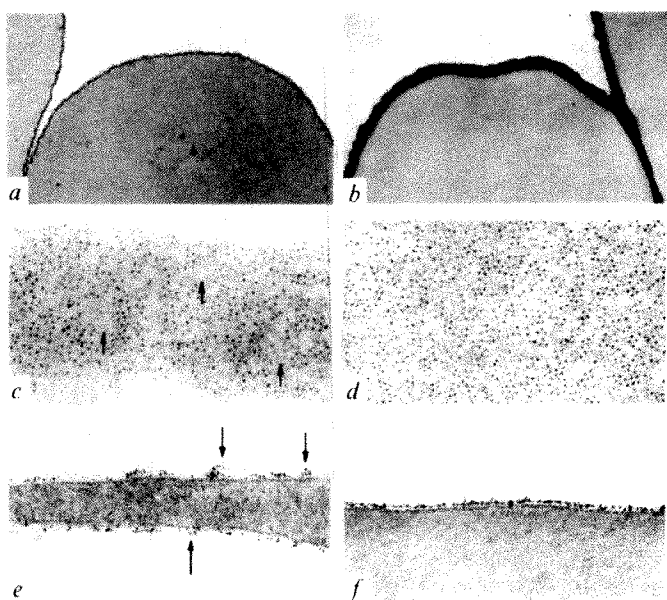


Fig. 1 Distribution of membrane probes on thin sections of thalassaemic (a, c, e) and normal (b, d, f) RBC. Thalassaemic RBC (a) stained with ruthenium red. Note the relatively thin layer of the reaction products when compared with normal RBC (b). Tangential section of thalassaemic RBC (c) treated with cationised ferritin (CF). The labelling pattern is interrupted by unlabelled areas (arrows). Quantification of the CF label on tangentially sectioned thalassaemic membranes showed reduction of about 40% compared with the uniformly CF-labelled normal RBC (d). Perpendicular section of thalassaemic RBC membrane (e) treated as follows: cells, washed in PBS, were reacted with 1 mM sodium periodate, washed twice, and treated with biotin hydrazide (2.5 mg ml⁻¹). Cells were then fixed for 30 min at room temperature with Karnovsky's fixative, labelled with ferritin-avidin conjugates (1 mg ml⁻¹ ferritin)^{6,7}, washed again, post-fixed with 1% osmium tetroxide and embedded in Epon 812. Perpendicular section of representative RBC membrane from healthy donors (f) treated in the same manner as in e. Note the presence of aggregates separated by unlabelled gaps in e, in addition to the distance (arrows) of some of the ferritin particles from the membrane surface. In contrast, a uniform labelling pattern is obtained on normal RBC membranes in f. Magnifications: a, b $\times 40,000$; c, d $\times 80,000$; e, f $\times 60,000$.

Table 1 Sialic acid content of thalassaemic and control RBC membranes

Membranes	($\mu\text{g mg}^{-1}$) Membrane protein
Control (9)	44.9 \pm 2.5
β -Thalassaemia major (17)	28.4 \pm 3.3
β -Thalassaemia intermedia (4)	27.3 \pm 4.1

The RBC membranes were prepared as described by Kahane and Rachmilewitz⁴. Sialic acids were determined by the thiobarbituric acid procedure¹³. The number of samples tested is indicated in parenthesis.

frequent blood transfusions¹⁰. Nevertheless, the distribution of SA on the RBC surface in these patients was similar to that seen on β -thalassaemia major RBC membranes (Fig. 1e).

The underlying mechanism for the observed reduction in surface SA in thalassaemic RBC is still unknown. Several factors could be responsible for this phenomenon. Membrane biogenesis may be defective in precursor cells, leading to the release of RBC with altered content and distribution of SA. Alternatively, reduced SA levels could be attributed to an enhanced rate of their removal from thalassaemic RBC membranes. Thus, the short life-span of thalassaemic RBC¹⁰ may be related to an increased rate of sequestration due to the loss in surface SA.

Recently, Riggs and Ingram¹² have also reported lower levels of SA in RBC membranes of patients with sickle cell anaemia. In view of the results recorded here, it is possible that a reduction in SA may occur in other types of anaemia. The potential interrelationship between the reduced sialic acid content in the membrane and RBC pathology warrants further investigation.

We thank Mr P. Benda for his support in this study and Fani Reichert for excellent technical assistance. E.A.R. is an established investigator of the Chief Scientist's Bureau, Ministry of Health, Israel.

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Intraventricular kainic acid preferentially destroys hippocampal pyramidal cells

THE hippocampus is particularly vulnerable to a variety of conditions, such as anoxia, status epilepticus and senile dementia, in which central neurones are lost^{1,2}. Most commonly, the lesion involves only the Sommer sector (h_1) and the endfolium (h_3 – h_5), sparing area h_2 , the fascia dentata and most regions outside the hippocampal formation. The consequences for hippocampal connections are unknown. Studies on the rat hippocampus suggest that connections made by the affected neurones could be replaced by axons of other neurones which project to the same areas^{3,4}. These anomalous synapses might either compensate in part for the loss of cells or contribute to whatever functional deficits may derive from the lesion. Since a good deal is known about afferent and efferent hippocampal connections in the rat, this animal might serve as a model for studies of hippocampal damage. However, the selective pathology seen clinically cannot be reproduced by conventional lesioning techniques. Ideally, one would like to use a toxin relatively specific for the neurones in question. Kainic acid, a potent excitatory analogue of glutamic acid^{5–7}, has been used to destroy neurones in the arcuate nucleus⁸ and striatum,^{9–11} while sparing fibres which pass to or through these regions. Previous workers have also briefly noted lesions in the hippocampus,^{8,11} but these were not described. Accordingly, we injected kainic acid intraventricularly into the rat brain and studied its effect on hippocampal neurones. We now report the unusual sensitivity of CA3–CA4, and to a lesser extent CA1, pyramidal cells to this agent. Our results suggest that kainic acid lesions can provide a model of hippocampal damage in man.

Male Sprague–Dawley rats (60–150-d-old) were anaesthetised with sodium pentobarbital and positioned in a stereotaxic apparatus (David Kopf Instruments) with the nose bar set at +5. A hole was drilled on bregma at 1.5 mm lateral to the midline, and a 1- μ l unbevelled Hamilton syringe was inserted to a depth of 4.7 mm from the surface of the skull. Kainic acid dissolved in 1 μ l of Elliott's artificial cerebrospinal fluid¹² (pH 7.3–7.5) was infused over a 30-min period. After 1–55 d the rats were killed by transcardial perfusion with neutralised formalin in normal saline, and the brains were examined histologically.

A modified Fink–Heimer method¹³ was used to detect degenerating neurones 1 and 3 d after kainic acid treatment. Argyrophilic (degenerating) hippocampal neurones (Fig. 1b) were seen at these survival times after unilateral injection of as little as 0.1 μ g (about 0.5 nmol). At this dose kainic acid killed only the pyramidal cells in area CA3a at the rostral pole of the hippocampus. Increasing doses destroyed pyramidal cells at increasingly caudal levels and in additional subfields. Cell destruction proceeded from the entrance of the fimbria (area CA3a) toward the dentate hilus (areas CA3c and CA4). At a dose of 0.8 μ g argyrophilic pyramidal cells were seen throughout areas CA3 and CA4 in all sections cut through the rostral half to two-thirds of the hippocampal formation (Fig. 1a). Intermediate patterns were obtained when 0.3 or 0.5 μ g was administered. Doses higher than 0.8 μ g were usually required to kill the pyramidal cells of area CA1, and the pyramids in area CA2 were seldom destroyed by any non-fatal dose (LD₅₀ of about 1.1 μ g). Dentate granule cells were destroyed only when 3 μ g of the drug was injected directly into the hippocampus. This hierarchy of sensitivity to kainic acid resembled that of hippocampal neurones to injurious conditions in man, and it could not be explained by the proximity of affected neurones to the lateral ventricle.

At 1 d after injection the degenerating elements were predominantly cell bodies and dendrites. By 3 d, however, we could identify a substantial number of degenerating axons and boutons. Kainic acid did not directly damage the septo-

hippocampal fibres (Fig. 2b) nor the hippocampal mossy fibres, whose cell bodies of origin remained intact. Thus, as in other regions,^{8,14,15} kainic acid selectively attacked cell bodies and dendrites, whereas axons of the same cells were only secondarily affected and afferent fibres were apparently unaffected.

In adjacent sections stained with cresyl violet degenerating cell bodies appeared severely shrunken, and their nuclei stained more darkly than normal (Fig. 1d). These neurones appeared to have been partially removed by 3 d after injection (Fig. 1c), although degenerating neurones could still be seen in some cases for at least 2 weeks. Over a period of several weeks areas of massive neuronal degeneration became gliotic and then atrophied (Fig. 2). Kainic acid exerted its toxic action directly on the hippocampus and not indirectly through an action elsewhere since the same histological results were obtained after intrahippocampal injection. That argyrophilic neurones actually degenerated and were not merely damaged reversibly was shown by the reduction or absence of neurones 1–2 months after injection in areas where argyrophilic cells were present at earlier times (Fig. 2a).

Argyrophilic neurones were not detected in regions other than hippocampus, when 0.1–0.3, and usually 0.5, μ g were administered, except for perhaps a few cells in the lateral septum immediately adjacent to the site of injection. Thus at these dosage levels kainic acid produced a fairly pure hippocampal lesion. With increasing dose, argyrophilic neurones were observed in additional regions, including some of the thalamic nuclei, amygdala and deep layers of the cerebral neocortex (but not striatum or hypothalamus). In man, cell loss in these regions often accompanies loss of hippocampal pyramidal cells.^{1,2} No degenerating cells were identified in areas known to project to the hippocampal formation, except in nucleus reuniens of the thalamus¹⁶ and in layer III of the entorhinal cortex.

Animals given intraventricular kainic acid showed symptoms of a partially suppressed convulsion lasting 3–4.5 h. At all doses

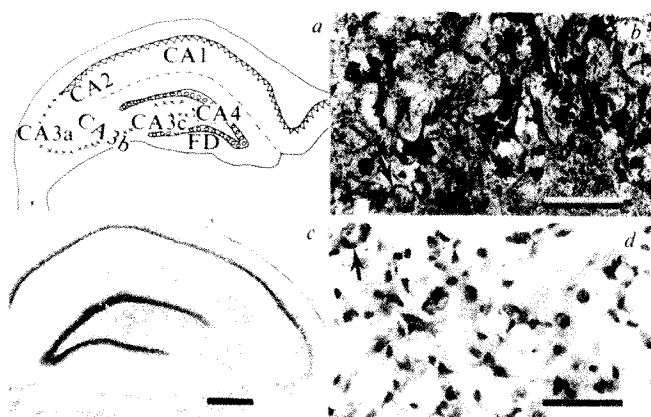


Fig. 1 Frontal sections through the dorsal rat hippocampal formation 3 d after ipsilateral intraventricular injection of 0.8 μ g of kainic acid. *a*, Diagram of hippocampal formation showing the location of degenerating pyramidal cells (x). The hippocampal formation is divided into fields according to Lorente de N \acute{o} . Area CA1 corresponds roughly to h_1 in man, CA2 to h_2 , CA3 to h_3 and h_4 and CA4 to h_5 . Dashed lines denote the hippocampal fissure and the border of areas CA1 and CA2. FD, fascia dentata; F, fimbria; triangles, normal-appearing pyramidal cells; circles, dentate granule cells. A few scattered normal-appearing neurones were also found among the degenerating cells. *b*, High power view of part of area CA3c showing degenerating pyramidal cells. A few normal-appearing neurones (arrow) are also present. Fink–Heimer stain. Scale bar, 0.05 mm. *c*, Adjacent section stained with cresyl violet showing neuronal loss in areas CA3 and CA4. Note the glial proliferation in these areas. Scale bar, 0.5 mm. *d*, High power view of part of area CA3c showing darkly-stained shrunken pyramidal cells. Small dark bodies are probably glial cells. Some normal-appearing neurones (arrow) remain. Cresyl violet stain. Scale bar, 0.05 mm.

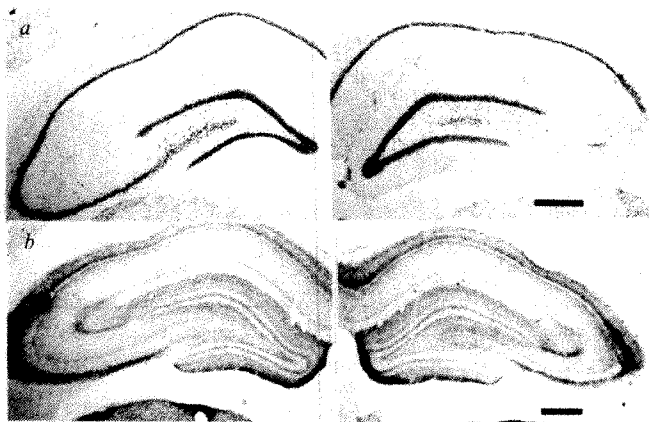


Fig. 2 Dorsal hippocampal formation 34 d after intraventricular injection of 0.8 µg of kainic acid. Injected side on the right, contralateral side on the left. Ipsilateral areas CA3 and CA4 have atrophied noticeably by this time. *a*, Cresyl violet stain to show permanent loss of pyramidal cells in areas CA3 and CA4. Note persistent gliosis in affected areas. Scale bar, 0.5 mm. *b*, Acetylcholinesterase stain to show preservation of septohippocampal fibres^{25,26}. The density and distribution of these fibres is unaltered by kainic acid. Timm's sulfide silver staining of mossy fibres²⁷ showed those fibres were also unaffected. Scale bar, 0.5 mm.

these included vibrissa tremor and distended eye ipsilateral to the injection and at doses of 0.8 µg and above there was in addition upper body tremor, rigidity of the limbs and foaming at the mouth. Several convulsants which interfere with GABA-mediated transmission and induce prolonged hippocampal seizures produce neuronal damage in baboons¹⁷⁻¹⁹ and cats²⁰ similar to that which kainic acid causes in rats. These agents probably induce a seizure through disinhibition, whereas kainic acid depolarises neurons directly. The similar pathology in these cases suggests that the cell loss in the present study resulted from a prolonged hippocampal seizure, similar to an episode of status epilepticus.

With regard to its mechanism of action, previous workers have assumed that kainic acid serves simply as a potent glutamate agonist, interacting with a glutamate receptor to effect a prolonged depolarisation. This chronic excitation would then lead to an irreversible ionic imbalance resulting in cell death. Indeed the destructive effects of the two amino acids on striatal and arcuate neurones appear very similar,^{8,10} although they are effective at doses about two orders of magnitude apart. In hippocampus, however, we have found glutamic acid to be virtually ineffective in destroying the pyramidal cells, even when it was injected directly into this region at a dose more than three orders of magnitude greater than the minimally effective dose of kainic acid. Furthermore, the dentate granule cells were quite resistant to the toxic effects of this agent, even though glutamate probably serves as a major excitatory transmitter in the fascia dentata²¹⁻²³ and the granule cells are readily depolarised by iontophoretically applied glutamate (unpublished observation). These results raise the possibility that either the glutamate receptors in the hippocampal formation differ from those in the striatum and arcuate nucleus or the destructive effects which kainic acid elicits in the hippocampus are not mediated through an interaction with a glutamate receptor.

Since kainic acid preferentially affects those neurones which are most susceptible to damage by a variety of adverse conditions, these lesions may provide a useful model of hippocampal injury in man. Kainic acid should prove advantageous in investigations of (a) the vulnerability of the hippocampus, particularly in status epilepticus, (b) the plasticity of hippocampal connections after brain damage and (c) the presently controversial behavioural role of the hippocampus.

We thank Miss J. Mueller for secretarial assistance. This

study was supported by grants from the National Science Foundation and the National Institutes of Health.

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Received October 3; accepted 21 November 1977.

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In vitro release of Leu- and Met-enkephalin from the corpus striatum

We have suggested previously that Met-enkephalin and Leu-enkephalin¹ may act as neurotransmitters in the central and peripheral nervous systems^{2,3}. Recent studies on the distribution of the enkephalins support this view⁴⁻⁶. An important step in establishing a possible neurotransmitter role of the enkephalins is the demonstration of their release from neuronal sites by potassium ions or veratridine. Indirect evidence for the release of enkephalin or endorphin has been provided by several workers⁷⁻¹⁰. We now present direct evidence for such release from isolated brain slices and synaptosomal preparations.

The experiments were carried out on preparations of the rabbit and guinea pig striatum, which contain very high endogenous levels of Met- and Leu-enkephalin. The enkephalins were isolated by adsorption on Amberlite CG-400 and elution with formic acid¹¹, followed by adsorption on, and elution with methanol from, Amberlite XAD-2 (ref. 3). All the samples were assayed on the mouse vas deferens with Met- and Leu-enkephalin as standards. Antagonism by naloxone (900 nM) was routinely used in each assay to check for specific opioid peptide activity.

A small basal release of enkephalin (0.4% of tissue content in 30 min) was detected in the superfusate from a layer of crude synaptosomes prepared from rabbit striatum (Table 1). When the potassium ion concentration of the superfusion fluid was increased from 5.94 to 50 mM, the amount of enkephalin recovered in a 15-min collection increased to 1.9% of the tissue content. A second perfusion, 30 min after the first, with Krebs solution containing 50 mM K⁺, produced a much smaller release of enkephalin. In a separate set of experiments, the concentration of calcium chloride was increased to 5.08 mM and the potassium-evoked release of enkephalin was more than double that in Krebs solution containing 2.54 mM calcium chloride. Omission of calcium chloride from the medium mar-

Table 1 Enkephalin release from rabbit striatal synaptosomes

Calcium chloride and potassium ion concentrations in Krebs solution (mM)		No. of experiments	Fractional release (%) during a collection period	
CaCl ₂	K ⁺		1st collection	2nd collection
2.54	5.94	11	0.4±0.2	0.2±0.15
5.08	5.94	4	0.5±0.2	—
2.54	50	4	1.9±0.3	0.5±0.15
5.08	50	4	4.4±0.2	0.9±0.2
0	50	3	0.8±0.1	—
0+EDTA (0.5 mM)	50	3	not detectable	—

A crude synaptosomal fraction (P₂) of rabbit striatum was prepared by homogenising (Teflon pestle and glass tube) 0.8–1.0 g of tissue in 0.32 M sucrose (1 g tissue per 10 ml sucrose). The nuclear fraction (10,000g min) was discarded and the P₂ fraction obtained by centrifuging the supernatant at 17,000g for 20 min. The P₂ pellet was resuspended in Krebs solution and layered on a 1-cm diameter filter superfused with Krebs solution at 36 °C, gassed with 95% O₂ and 5% CO₂. The preparation was superfused for 20 min before collecting the superfusate. Basal release at 2.54 mM CaCl₂ and 5.94 K⁺ was determined in 30-min periods and potassium-evoked release in 15-min collection periods. Fractional release refers to the total enkephalin activity (measured as Met-enkephalin) released during a collection period into the superfusate and is expressed as % of the tissue content. In some experiments a second collection of superfusate was made 30 min after the first collection. All determinations were made in separate experiments, apart from the second collections. The limit of sensitivity of the assay was 0.5 ng. The values are the means±s.e.m.

kedly reduced the evoked release of enkephalin; the addition of EDTA to the calcium-free medium reduced the release to undetectable levels.

It has been suggested that Met-enkephalin is merely a proteolytic breakdown product of β -endorphin¹². Experiments were carried out to test the possibility that the source of the released enkephalin might be the larger endorphins released into the medium and subsequently degraded, rather than the direct release of enkephalin. In three perfused synaptosomal preparations, β -endorphin was added to the superfusion medium so that 1 nmol of the peptide passed through the layer of synaptosomes in 15 min. There was no detectable increase in enkephalin above basal levels in each of these experiments. The breakdown of only 0.1% of the β -endorphin to Met-enkephalin would have been detected (sensitivity of assay, 0.5 ng Met-enkephalin), and so we conclude that cleavage of β -endorphin does not occur to any significant extent under our experimental conditions.

The effect of potassium as a depolarising agent is not specific for neurones. Veratridine may be considered a more specific stimulus¹³, and therefore we tested the effect of this agent on enkephalin release from superfused slices of the guinea pig striatum. Preliminary experiments established that the release by veratridine was small and variable, which was considered to be due to enzymatic breakdown of enkephalin. A series of di- and tripeptides were screened for their ability to reduce breakdown. It was found that a combination of leucyl-leucine, leucyl-glycine and tyrosyl-tyrosine (each 1 mM) prevented the breakdown of both Met- and Leu-enkephalin when incubated with either brain homogenates or striatal slices. The inhibition by the dipeptides decreased with time, suggesting

that the mechanism of action might be substrate competition. Control experiments showed that the recovery of added Met-enkephalin from superfused striatal slices increased from 47±5% (*n* = 5) in the absence of the dipeptides to 97±11% (*n* = 5) in their presence.

In the presence of the dipeptides, no basal release of enkephalin (<0.5 ng) could be detected in 10-min collections of striatal superfusate. The addition of veratridine (50 μ M) to the superfusing fluid for 2–10 min caused a release of enkephalin. During a prolonged exposure to veratridine (10 min), the output of enkephalin increased over the first 6 min and then declined (Fig. 1a). When slices were exposed to veratridine for 3 min, at intervals of 30 min, the output of enkephalin declined with successive exposures (Fig. 1b). A similar phenomenon was seen with potassium-evoked enkephalin release from synaptosomes. This decrease in output with successive exposures to veratridine may be due to incomplete recovery of the tissue from the preceding intense stimulation, depletion of the releasable store of enkephalin, or a decrease in the viability of the slices during the course of the experiment. Tetrodotoxin (1.7 μ M) abolished the veratridine-induced output of enkephalin (Fig. 1b).

In another series of experiments, the material released by veratridine was analysed by thin layer chromatography to separate Met- and Leu-enkephalin (Table 2). Both peptides were found to be present in the superfusate and the tissues. The ratio of Met-enkephalin to Leu-enkephalin released from the guinea pig striatum was very similar to the ratio of these peptides present in the tissue.

Our results from striatal synaptosomes and slices indicate that the enkephalins, in common with other putative neurotransmitters, are released from brain tissue by an increase in

Table 2 Comparison of stimulated output and tissue content of Met-enkephalin and Leu-enkephalin

	No. of experiments	Met-enkephalin (ng)*	Leu-enkephalin (ng)*	ratio Met/Leu†
stimulated output	6	5.5±0.9	1.7±0.16	3.4±0.5
tissue content	4	79±22	20±5	3.8±0.2

Guinea pig striatal slices (600- μ m thick, sagittal section) from two striata weighing approximately 300 mg were used in each experiment. The slices were superfused (1.1 ml min⁻¹) for 45 min with Krebs solution at 36 °C and gassed with 95% O₂ and 5% CO₂. The slices were exposed to veratridine (50 μ M) for 10 min and the superfusate collected for 20 min from the start of exposure to veratridine. The dipeptides, Leu-Leu, Tyr-Tyr and Leu-Gly (each 1 mM) were present in the superfusing fluid for 5 min before exposure to veratridine and throughout the collection period. For the determination of endogenous enkephalins, the tissues were homogenised in 0.1 M HCl. All samples (superfusate and tissues) were chromatographed on Amberlite CG-400 and XAD-2 columns, then spotted on 0.25-mm silica gel plates and developed with ethyl acetate:pyridine:water:acetic acid:ethane-1,2-dithiol (100:44:25:11:0.2). The marker lanes were stained with cadmium-ninhydrin reagent, and the areas corresponding to Met-enkephalin (*R_f* \approx 0.4) and Leu-enkephalin (*R_f* \approx 0.55) were removed, eluted and assayed. The values are the means±s.e.m.

*Uncorrected for recovery from all chromatographic procedures; this was estimated to be 30–35%.

†Ratios calculated from individual experiments.

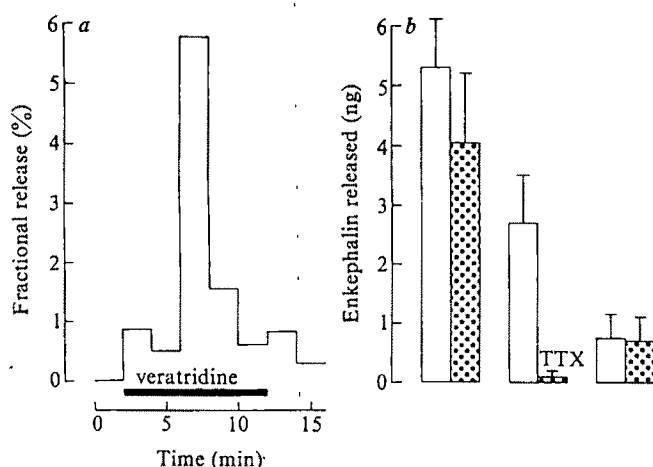


Fig. 1 Release of enkephalin from guinea pig striatal slices. The slices were superfused (1.1 ml per min) for 45 min with Krebs solution at 36 °C and gassed with 95% O₂ and 5% CO₂. *a*, 600 μ m thick sagittal sections from two striata weighing approximately 300 mg were exposed to veratridine (50 μ M) for 10 min. The superfusate was collected at 2-min intervals. Fractional release refers to the total enkephalin activity (assayed as Met-enkephalin) released into the superfusate expressed as a % of the tissue content. *b*, 450 μ m thick sagittal sections from one striatum weighing approximately 150 mg were exposed to veratridine (50 μ M) for 3 min every 30 min. The superfusate was collected for 12 min from the start of veratridine exposure. The open columns represent the output of enkephalin on three successive exposures to veratridine. The stippled columns represent the output of enkephalin from tissues in which the second exposure to veratridine was made in the presence of 1.6 μ M tetrodotoxin (TTX). All outputs were assayed as Met-enkephalin. Each column represents the means from three experiments, the vertical bar indicating s.e.m. In both *a* and *b*, Leu-Leu, Tyr-Tyr and Leu-Gly (each 1 mM) were present in the superfusing fluid for 5 min before exposure to veratridine and throughout the collection periods. All samples (superfusate and tissues) were chromatographed on only Amberlite CG-400 and XAD-2 columns with a recovery of 90–100%. No attempt was made to separate Leu-enkephalin from Met-enkephalin.

potassium ions or veratridine. The potassium-induced release is calcium-dependent and the effect of veratridine is abolished by tetrodotoxin. We suggest that the source of this evoked release is the neuronal stores of enkephalins and that the present results provide additional direct evidence that Met- and Leu-enkephalin may act as central neurotransmitter agents.

This work was supported by grants (to H.W.K.) from the US National Institute on Drug Abuse, the MRC and the US Committee on Problems of Drug Dependence.

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Received 31 October 1977; accepted 9 January 1978.

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Release of enkephalin from rat globus pallidus *in vitro*

SINCE the discovery of the endogenous opioid peptides Leu- and Met-enkephalin in mammalian brain, there has been considerable interest in their possible physiological functions^{1–3}. Recent immunohistochemical studies have shown that the enkephalins are localised in neurones in various regions of the central nervous system, with high concentrations in nerve terminals^{4,5}. These morphological studies and the results of detailed regional analyses of rat brain by radioimmunoassay have indicated that enkephalin-containing nerve terminals are particularly concentrated in the rat globus pallidus, which by radioimmunoassay has been found to contain a concentration of enkephalin six to eight times higher than that observed in any other brain region^{6,7}. Here, we describe a calcium-dependent evoked release of enkephalin-like immunoreactivity from slices of rat globus pallidus *in vitro*. This finding supports the view that enkephalins may be released as neurotransmitter substances from nerve terminals in the brain.

Globus pallidus tissue was dissected from each side of thick frontal sections of chilled rat brain, using the anatomical landmarks of the atlas of König and Klippel⁸. The pooled samples of tissue from left and right sides weighed approximately 30 mg and were contaminated with small amounts of adjacent striatal and thalamic tissue. The tissue from each brain was cross-cut at 200- μ m intervals with a McIlwain tissue chopper, and the resulting slices were suspended in Krebs-bicarbonate medium in a small plastic superfusion chamber at 37 °C, as described previously^{9,10}. The slices were superfused with a modified Krebs-bicarbonate medium at 37 °C, containing bovine serum albumin and bacitracin to protect against loss of released enkephalin by absorption or metabolism. After an initial wash period of 13 min, superfusate samples were collected at 3-min intervals and aliquots were analysed for enkephalin using a sensitive radioimmunoassay procedure^{11,12}. This assay involved the use of an anti-Leu-enkephalin rabbit serum for which Leu-enkephalin had an affinity some 30 times higher than that of Met-enkephalin. It was thus not possible to distinguish between Leu- and Met-enkephalin, and the results were calculated in terms of units of enkephalin (1 unit = 1 ng Leu-enkephalin equivalent). Since rat globus pallidus is reported to contain a sixfold preponderance of Met-enkephalin over Leu-enkephalin⁷, the observed immunoreactivity was probably due to a mixture of the two peptides.

A rapid release of enkephalin-like immunoreactivity was observed during the initial 13-min wash-out period; after this, however, the spontaneous release of enkephalin decreased to 0.1–0.2% of the total tissue stores per minute. In several instances, the concentration of enkephalin present in such spontaneous release samples was less than 10 pg ml⁻¹, which represented the lower limit of accurate measurement for the radioimmunoassay procedure. During exposure to a high potassium medium (K⁺ 50 mM) for a 6-min period, there was a large increase in the rate of release of enkephalin-like immunoreactivity (Fig. 1a). During the period of potassium ion stimulation the rate of enkephalin release was 10–20 times higher than in the preceding spontaneous release samples, and a total of 9.6 \pm 1.8% of the total tissue enkephalin content was released during the 6-min stimulation period (mean \pm s.e.m., *n* = 10 experiments). On return to normal medium the efflux of enkephalin decreased rapidly to resting values similar to those observed before the stimulus. The release of enkephalin was very rapid during the initial 3 min of the period of potassium ion exposure, and was significantly slower during the second 3 min of the potassium ion pulse (Fig. 1). Further evidence for an exhaustion of the tissue stores of releasable enkephalin was obtained from experiments in which the tissue slices were exposed to a second potassium ion pulse 9 min after the first exposure (Fig. 2). The second exposure to potas-

sium ion released less than half as much enkephalin as the first, although the tissue content of enkephalin at the beginning of the second stimulation period was only some 10% lower than at the beginning of the first period of stimulation.

In the absence of calcium in the superfusion medium, the spontaneous release of enkephalin occurred at a low rate, similar to that observed in control experiments, and high potas-

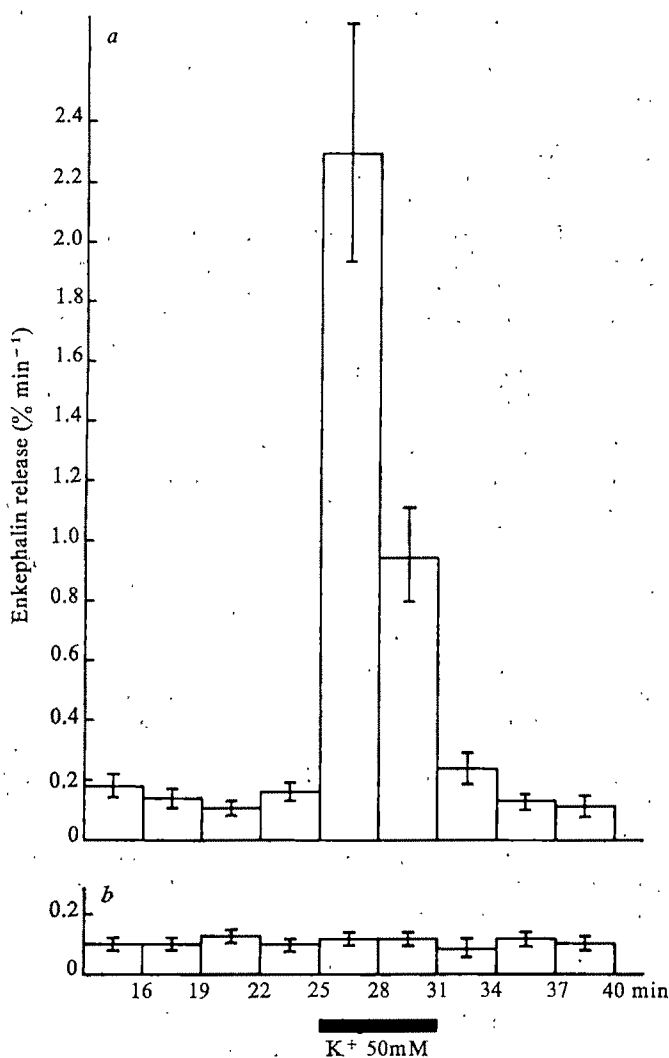


Fig. 1 Release of enkephalin immunoreactivity from slices of rat globus pallidus superfused with normal (a) or calcium-free medium (b). The slices of pallidal tissue (about 30 mg wet weight) were superfused at a rate of approximately 300 μ l min⁻¹, and after an initial wash period of 13 min, samples of the superfusate were collected at 3-min intervals for subsequent radioimmunoassay. The superfusion medium was a modified Krebs-bicarbonate solution with the following composition: NaCl 127 mM; KCl 3.73 mM; CaCl₂ 1.8 mM; KH₂PO₄ 1.18 mM; MgSO₄ 1.18 mM; NaHCO₃ 20 mM; D-glucose 2 g l⁻¹. The medium was gassed with an oxygen/carbon dioxide (95%:5%) mixture, and contained bovine serum albumin (Sigma, crystalline) (0.1%, w/v) and the peptidase inhibitor bacitracin (Sigma) (30 μ g ml⁻¹). Twenty microlitres of a saturated solution of Na₂EDTA was added to each of the tubes used to collect the superfusate samples as a further precaution to protect released enkephalin from enzymatic degradation. During the period indicated by the horizontal black bars the slices were exposed to a medium in which KCl was substituted for NaCl to give a final K⁺ concentration of 50 mM. One hundred microlitre aliquots of the superfusates were analysed for enkephalin using a radioimmunoassay procedure¹¹. Results are expressed as percentage total tissue enkephalin released per minute, based on measurements of the total tissue enkephalin content at the end of the superfusion (Table 1). This was estimated after extraction of the tissue with boiling 1 M acetic acid, homogenisation and neutralisation of the centrifuged extracts, as described previously¹¹. Control values (a) represent means \pm s.e.m. for 10 experiments; results obtained with calcium-free medium (b) are means \pm s.e.m. for four experiments.

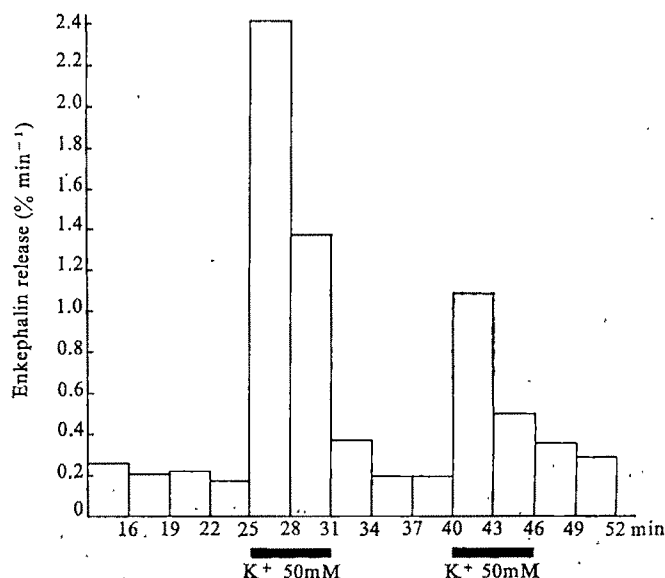


Fig. 2 Release of enkephalin from rat globus pallidus slices in response to two periods of exposure to high potassium medium. Mean values for two experiments; conditions as in Fig. 1a.

sium ion exposure was completely without effect in stimulating enkephalin release (Fig. 1b).

Measurements of the total enkephalin-like immunoreactivity in extracts of freshly dissected globus pallidus samples (Table 1) confirmed that this area of rat brain contains a very high concentration of enkephalin^{6,7}, amounting to 377 ± 15.4 units per g wet weight (mean \pm s.e.m., $n = 4$), a value some three to four times higher than that observed in rat striatum or hypothalamus in a previous regional survey using the same radioimmunoassay procedure¹². After a 40-min period of superfusion *in vitro*, the total enkephalin immunoreactivity of the tissue slices was markedly reduced when compared with the values obtained from fresh samples of globus pallidus tissue, indicating that some 60% of the total tissue enkephalin content was lost during the course of the experiments (Table 1). This is in contrast to the results obtained in analogous experiments with the other neuropeptides, substance P^{9,10} or somatostatin and neurotensin (L.L.I., S.D.I., F.E.B., M. Brown and W. Vale, unpublished), in which only a small proportion of the tissue stores of peptides were lost during a similar period of superfusion. The loss of tissue enkephalin during superfusion could not all be accounted for by the amounts of free peptide measured in the superfusate samples. During the 40-min superfusion period, approximately 25% of the total tissue enkephalin content was recovered as immunoreactive material in the superfusate samples; the remaining substantial loss of tissue enkephalin must presumably be attributed to enzymatic degradation, which was only partially inhibited by the addition of bacitracin¹³. The importance of adding this peptidase inhibitor was illustrated by the results of experiments in which bacitracin was

Table 1 Tissue content of enkephalin immunoreactivity in rat globus pallidus

Conditions	No. of experiments	Tissue enkephalin (units per globus pallidus sample)
Freshly dissected	4	11.32 ± 0.46
At end of 40-min control superfusion	10	4.62 ± 0.38
At end of 40-min superfusion in calcium-free medium	4	4.06 ± 0.52
At end of 40-min superfusion with control medium without bacitracin	4	4.17 ± 0.53

omitted. In these conditions, the spontaneous release of enkephalin was so low that it could not be measured accurately by the radioimmunoassay procedure in a majority of the resting efflux samples. Furthermore, although exposure to high potassium ion evoked a measurable release of enkephalin, the amounts collected during the 6-min period of exposure to high potassium ion represented only $5.8 \pm 1.5\%$ of the total tissue stores (mean \pm s.e.m., $n = 4$), significantly lower than the values observed in response to high potassium ion in media containing bacitracin. The tissue content of enkephalin at the end of these experiments, however, was not significantly different, whether or not bacitracin had been added to the medium (Table 1), suggesting that protection from enzymatic degradation is not important for stabilising enkephalin in its intracellular storage sites, but is critical in inhibiting degradation after the peptide has been released.

Because of this degradation, the observed percentage of enkephalin released should be viewed with some reservation. Since our antisera read both enkephalins, more detailed analysis of the release and stability of each enkephalin in these experimental conditions will be needed in order to arrive at more accurate estimates of the percentage of the total enkephalin content which can be released by potassium *in vitro*. Preliminary experiments indicate that the rate of catabolism of Met-enkephalin within the perfusion chambers is faster than that of Leu-enkephalin⁵. Nevertheless, the overall results of the present experiments have been validated in a variety of experimental conditions, with controls for release and peptidase inhibition.

In summary, the present results show that enkephalin can be released from rat brain *in vitro* in response to depolarisation caused by elevation of the external potassium ion concentration, and that this evoked release is completely dependent on the presence of calcium ions in the external medium. Such behaviour is characteristic of a number of other substances thought to be released as neurotransmitters in the central nervous system¹⁴⁻¹⁶, and strongly supports a neurotransmitter role for enkephalin in mammalian brain. A similar calcium-dependent potassium-evoked release of enkephalin has been described from rat brain synaptosome preparations¹⁷ and from rabbit guinea pig striatal tissue¹⁸.

F.E.B. was supported by a grant from the William Randolph Hearst Foundation and by grant no. DA-01785.

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Received 22 November; accepted 9 December 1977.

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Inhibition of hepatitis B DNA polymerase by intercalating agents

INFECTION with hepatitis B virus (HBV) is widespread and increasing in incidence in many countries. Unfortunately, there is as yet no effective chemotherapy. The hepatitis B virion is unique in that it contains both circular DNA and a DNA polymerase enzyme (for review see ref. 1). After the description of the enzyme² in concentrated preparations of hepatitis B antigen (HBsAg) containing Dane particles it was shown that enzyme activity was inhibited by intercalating agents such as ethidium bromide³. We have extended these early studies to include intercalating agents that can be safely administered to patients. We show here that several intercalating agents in clinical use inhibit the hepatitis B (HB) DNA polymerase reaction *in vitro* and we discuss the implications of these findings for the therapy of hepatitis B infection.

The following compounds were included in the study: ethidium bromide (molecular weight 394.3), chloroquine diphosphate (MW 515.9), primaquine diphosphate (MW 455.4), quinacrine hydrochloride (MW 472.9), methylene blue (MW 319.9), chlorpromazine (MW 355.3), quinine hydrochloride (MW 360.9) (all from Sigma) and hydroxychloroquine (MW 530, Sterling-Winthrop). Ethidium bromide and chloroquine have been shown to intercalate by changes induced in the superhelical density of the replicative form of Φ X174 DNA (ref. 4). Quinacrine, chlorpromazine, methylene blue and quinine change the superhelical density of PM-2 DNA in the manner of intercalators when examined by viscometric titration⁵. Primaquine has been shown to change the superhelical density of PM-2 DNA in the manner of intercalators at low ionic strength⁶.

Pellets of HBsAg containing Dane particles were prepared from patients' sera and shown to contain hepatitis B surface antigen (HBsAg) and hepatitis B core antigen (HBcAg) by methods described previously⁶. Assays for HB DNA polymerase were carried out in reaction mixture containing 0.15 M KCl and 0.01 M MgCl₂ (ref. 7), and in the differential assay mix⁸ containing 0.4 M KCl and 0.04 M MgCl₂ as previously described^{2,6,7}. The DNA product of the reaction was shown to be associated with HBcAg (ref. 6). Concentrated stock solutions of the drugs were prepared in distilled water and aliquots were added to the reaction mixtures to give the desired concentrations. The concentration of intercalating agent resulting in 50% inhibition (IC₅₀) of the HB DNA polymerase reaction was calculated from the results by a modification of the method of Reed and Muench⁹ where applicable.

Ethidium bromide, the archetype intercalating agent, was the most effective inhibitor of hepatitis B DNA polymerase amongst the drugs examined. The DNA polymerase reaction was inhibited by 90% at a concentration of $10 \mu\text{g ml}^{-1}$ (2.5×10^{-6} M) of ethidium bromide in reaction mixture containing 0.15 M KCl and 0.01 M MgCl₂ (Fig. 1a); the IC₅₀ was $3.2 \mu\text{g ml}^{-1}$ (8.1×10^{-6} M). Higher concentrations of magnesium ion interfered with inhibitory effect of ethidium bromide. Thus in 0.04 M MgCl₂ only 77% of the DNA polymerase reaction was inhibited at a concentration of $25 \mu\text{g ml}^{-1}$ of ethidium bromide and the IC₅₀ was $10 \mu\text{g ml}^{-1}$ (Fig. 1a). Inclusion of the non-ionic detergent Nonidet P-40 (NP-40) in the reaction mixture did not affect the inhibitory action of ethidium bromide on HB DNA polymerase.

The other intercalators studied, although not as toxic as ethidium bromide, are much weaker intercalators and were weaker inhibitors of HB DNA polymerase. However, several of the drugs inhibited HB DNA polymerase substantially at concentrations achievable in human liver⁹. Chloroquine, the mainstay of malarial prophylaxis, inhibited the DNA polymerase reaction by 45 and 53% at concentrations of 250 and $500 \mu\text{g ml}^{-1}$ respectively (Fig. 1b); the IC₅₀ was $480 \mu\text{g ml}^{-1}$ (9.30×10^{-4} M; Table 1). Quinacrine was a more effective inhibitor of HB DNA polymerase than chloroquine

(Fig. 1b); the IC_{50} for quinacrine was $334 \mu\text{g ml}^{-1}$ ($7.1 \times 10^{-4}\text{M}$; Table 1). Inclusion of NP-40 in the reaction mixture at a concentration of 0.3% interfered with the inhibition of the DNA polymerase reaction by chloroquine (Fig. 1b); NP-40 also interfered with the inhibition of the enzyme by quinacrine. Magnesium ion reduced the inhibitory activity of chloroquine (Fig. 1b); thus at a concentration of chloroquine of $500 \mu\text{g ml}^{-1}$ only 16% of the reaction was inhibited in the presence of 0.03 M MgCl_2 (Fig. 1b). Primaquine, another antimalarial

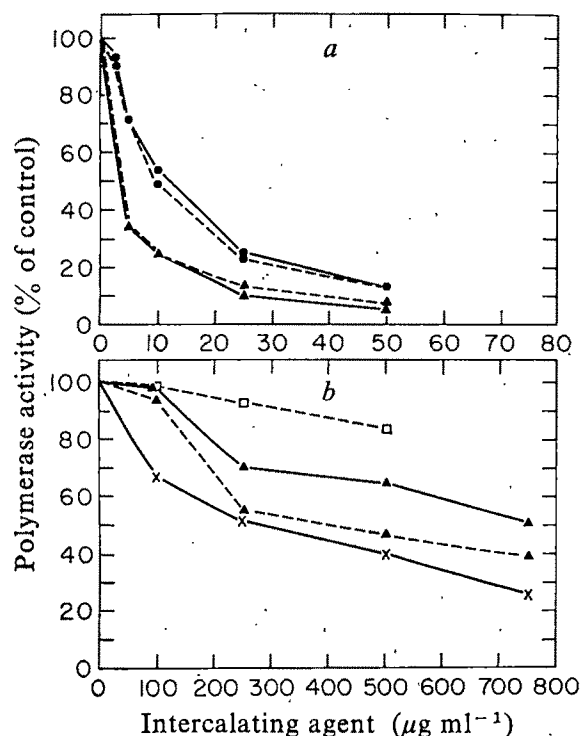


Fig. 1 Inhibition of hepatitis B DNA polymerase by intercalating agents. Pellets containing particulate HBAG, including Dane particles, were prepared by diluting serum 1:2 with phosphate-buffered saline (PBS), and centrifuging at $39,000g$ at 4°C , as previously described^{2,6}. The resulting pellet was washed in an equal volume of PBS or centrifuged through 5–20% sucrose gradients in PBS⁶. The final pellets were taken up in PBS in 1:20 of the original volume of serum. The standard DNA polymerase reaction mixture^{6,7} contained 0.05 M Tris-HCl, 20 mM dithiothreitol (DTT), 1 mM each of dATP, dCTP, and dGTP, and $2.5 \mu\text{Ci}$ of ^3H -dTTP at pH 7.5 in a final total volume of 0.1 ml. KCl, MgCl_2 , and NP-40, were included in the reaction mixture at various concentrations. The complete reaction mixture, including DNA polymerase-containing pellet suspension, was incubated at 38°C for 180 min. Enzyme activity was measured by the incorporation of ^3H -dTTP into acid-insoluble product^{6,7}. The ordinate represents enzyme activity expressed as % of control reactions not containing intercalators and the abscissa represents the concentration of intercalators in $\mu\text{g ml}^{-1}$. The same quantity of polymerase-containing pellet suspension was included in each reaction mixture. *a*, Ethidium bromide: ●—●, reaction mixture contained 0.4 M KCl, 0.04 M MgCl_2 and 0.3% NP-40; ●--●, same reaction mixture without NP-40; ▲—▲, reaction mixture contained 0.15 M KCl and 0.01 M MgCl_2 with 0.3% NP-40; ▲--▲, same reaction mixture without NP-40. Control reaction mixtures without intercalators in 0.04 M MgCl_2 with 0.3% NP-40 incorporated 2,200 c.p.m. of ^3H -dTTP per 0.1 ml reaction mixture into DNA; without NP-40 incorporation was 1,250 c.p.m. Control reactions without intercalators in 0.01 M MgCl_2 with NP-40 incorporated 1,100 c.p.m. of ^3H -dTTP and 875 c.p.m. without NP-40. Incorporation of 100 c.p.m. represents 4×10^{-3} pmol of ^3H -dTTP. *b*, ▲—▲, Chloroquine in reaction mixture containing 0.15 M KCl, 0.01 M MgCl_2 and 0.3% NP-40; ▲--▲, same reaction mixture with chloroquine but without NP-40. □, Chloroquine in reaction mixture containing 0.15 M KCl, 0.03 M MgCl_2 without NP-40. ×, Quinacrine in reaction mixture containing 0.15 M KCl, 0.01 M MgCl_2 without NP-40. Incorporation of ^3H -dTTP in control reactions without intercalators is given in (a) except that control reactions in 0.15 M KCl, 0.03 M MgCl_2 incorporated, 1,048 c.p.m.

agent, was a weak inhibitor of HB DNA polymerase; the IC_{50} of primaquine was $900 \mu\text{g ml}^{-1}$ ($2 \times 10^{-3}\text{M}$; Table 1). On the other hand, chlorpromazine was a very active inhibitor of the DNA polymerase reaction with an IC_{50} of $250 \mu\text{g ml}^{-1}$ ($7 \times 10^{-4}\text{M}$). Hydroxychloroquine and quinine hardly inhibited the enzyme reaction. However, methylene blue was a potent inhibitor of HB DNA polymerase; 94% of the reaction was inhibited at a concentration of $250 \mu\text{g ml}^{-1}$ of methylene blue ($7.8 \times 10^{-4}\text{M}$).

The inhibitory activity of combinations of intercalating agents against HB DNA polymerase was examined in order to determine whether additive or even synergistic effects would be observed. The combination of chloroquine and primaquine yielded moderately additive inhibitory effects on HB DNA polymerase (Table 2). Thus the combination of chloroquine at $500 \mu\text{g ml}^{-1}$ with primaquine at $250 \mu\text{g ml}^{-1}$ inhibited the DNA polymerase reaction by 66%, whereas at these concentrations each drug alone inhibited the reaction by 50 and 15%, respectively. However, the combination of chloroquine and primaquine at $500 \mu\text{g ml}^{-1}$ for each drug was not much more inhibitory than the lower concentrations (Table 2). The combination of chloroquine and quinacrine also showed moderately additive inhibitory activity against HB DNA polymerase. The combination of $500 \mu\text{g ml}^{-1}$ of chloroquine with 250 and $500 \mu\text{g ml}^{-1}$ quinacrine inhibited the DNA polymerase reaction by 71 and 78%, respectively (Table 2). In general the additive inhibitory effect of chloroquine combined with either primaquine or quinacrine was not much greater than the inhibition shown by the more active agent alone. The combination of chloroquine and chlorpromazine was quite effective in inhibiting the DNA polymerase reaction: $500 \mu\text{g ml}^{-1}$ of each drug combined inhibited the enzyme by 81% (Table 2). Chloroquine with methylene blue was an extremely potent inhibitory combination against HB DNA polymerase. Chloroquine at $500 \mu\text{g ml}^{-1}$ combined with 250 and $500 \mu\text{g ml}^{-1}$ of methylene blue inhibited the DNA polymerase reaction by 95 and 99.7%, respectively (Table 2). Primaquine combined with quinacrine also showed additive inhibitory effects against HB DNA polymerase (Table 2). The combination of quinacrine and chlorpromazine was extremely potent in inhibiting the DNA polymerase reaction; quinacrine $500 \mu\text{g ml}^{-1}$ combined with $100 \mu\text{g ml}^{-1}$ chlorpromazine inhibited the reaction by 84% (Table 2). Thus although the combinations of intercalators examined did not show synergistic effects, additive inhibitory effects were shown for all the drug combinations examined.

Of the three major antimalarial drugs examined primaquine was a much weaker inhibitor of HB DNA polymerase than either chloroquine or quinacrine. Primaquine seems to act as a preferential inhibitor of protein biosynthesis by causing a rapid breakdown of ribosomes when studied *in vivo*^{10,11}. Primaquine at $6 \times 10^{-4}\text{M}$ inhibits protein synthesis almost completely in *Bacillus megaterium* but has only marginal effects on the biosynthesis of DNA and ribonucleic acid¹⁰. The two phenothiazine derivatives, chlorpromazine and methylene blue, were quite active in inhibiting HB DNA polymerase. Careful study of the binding of these two compounds to DNA, in conditions selected to avoid precipitation of the DNA by the phenothiazine derivatives, by Allison and Hahn⁶ showed that these two drugs

Table 1 Comparison of inhibitory activity of intercalators against hepatitis B DNA polymerase

Intercalator	IC_{50} ($\mu\text{g ml}^{-1}$)
Ethidium bromide	3.2
Chloroquine	480
Quinacrine	334
Primaquine	900
Chlorpromazine	250

Reaction mixtures contained 0.15 M KCl and 0.01 M MgCl_2 without NP-40. Experimental details are given in the text and legend to Fig. 1.

Table 2 Inhibition of hepatitis B DNA polymerase by combinations of intercalators

CQ	Combinations of intercalators ($\mu\text{g ml}^{-1}$)				Enzyme activity (% of control)
	PQ	QC	CP	MB	
750	100	—	—	—	33
500	100	—	—	—	47
500	250	—	—	—	34
500	500	—	—	—	31
250	100	—	—	—	67
250	250	—	—	—	60
100	100	—	—	—	73
500	—	100	—	—	37
500	—	250	—	—	29
500	—	500	—	—	22
250	—	250	—	—	46
500	—	—	100	—	31
500	—	—	250	—	28
500	—	—	500	—	19
500	—	—	—	250	5
500	—	—	—	500	0.3
—	500	250	—	—	38
—	500	500	—	—	28
—	—	500	100	—	16
—	—	500	250	—	10

Reaction mixtures contained 0.15 M KCl and 0.01 M MgCl_2 without NP-40. Reaction mixtures included the combination of drugs at the concentrations shown. Other experimental details as detailed in the text and in the legend to Fig. 1. CQ, chloroquine; PQ, primaquine; QC, quinacrine; CP, chlorpromazine; MB, methylene blue.

did bind to DNA in the manner of intercalators. Interestingly, neither quinine nor hydroxychloroquine were active inhibitors of the DNA polymerase. The observation that the various combinations of chloroquine, primaquine and quinacrine were only moderately more inhibitory than the more active drug in the combination suggested that the drugs all attach to the same site on the DNA. Inhibition of HBV DNA polymerase by the intercalators decreased as the concentration of MgCl_2 was increased. Magnesium interferes with the binding of intercalators to DNA⁴.

Our results show that hepatitis B DNA polymerase can be inhibited by drugs in clinical use. It has been suggested that the DNA polymerase of the hepatitis B virion plays an important part in the replicative cycle of this virus in the hepatocyte¹. Thus, our findings suggest a new approach to the therapy of HBV infection using intercalating agents either singly or in combination. Drugs such as chloroquine and quinacrine accumulate in very high concentrations in the human liver during therapy⁹, and this pharmacologic property may make it feasible to treat HBV infection, even though high concentrations are required to inhibit HBV DNA polymerase. Furthermore, the possible therapeutic effects of these template blocking agents may be examined in combination with DNA synthesis inhibitors or with interferon¹². Interestingly, intercalating agents have the singular property of eliminating bacterial plasmids^{13,14}. Thus it may be speculated that these compounds may remove and thus 'cure' the infected hepatocyte from HBV DNA. Clinical studies to evaluate the therapeutic possibilities of intercalating agents in human HBV infection are under way.

We thank Drs Salvatore Vernace, Fenton Schaffner and Martin Goldfield for providing sera from patients infected with HBV.

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Received 1 August; accepted 9 December 1977.

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Relationship between β converting and γ non-converting corynebacteriophage DNA

BACTERIOPHAGE can convert non-toxin producing strains of *Corynebacterium diphtheriae* to toxinogeny, and the significance of the difference between β -tox⁺ converting corynebacteriophage isolated by Freeman¹ and γ -tox non-converting phage isolated in this laboratory² has been studied sporadically over the past 20 yr. These two phages are serologically related, but are heteroimmune when tested reciprocally against their lysogenic derivatives³. Both phages exhibit similar one-step growth characteristics and patterns of inducibility by ultraviolet light⁴ and are morphologically similar though differing slightly in head and tail measurements⁵. The phages recombine vegetatively, though at a frequency lower than in homoimmune β matings⁶ and also recombine as prophages in tandem double lysogens⁷. It has been shown⁸ that γ non-converting phage probably carries a cryptic tox gene, or part of the gene, as matings between β -tox and γ -tox have yielded tox⁺ recombinants. These data all suggest that there is a high degree of genetic homology between these two phages. To assess this point more directly we have examined DNA heteroduplexes of the phages and DNA fragments produced by various restriction endonucleases. The data show that the two phages are almost identical.

Heat-inducible mutants of β and γ phages, β -tsr3 and γ -tsr1, isolated in this laboratory⁹ were used for the production of purified phage DNA. These mutants are unlikely to vary significantly from wild-type phage because both are conditional lethals, presumably a result of point mutations and thus not detectably different from the wild type in heteroduplex analyses. Lysogens of these phages in strain C7 of *C. diphtheriae* were induced in tryptose yeast extract broth¹⁰ supplemented with 0.2% Tween 80. The phage were concentrated directly from lysates by high-speed centrifugation (100,000g) following an initial low-speed cycle (8,000g). The pellets were immediately resuspended in 0.05 M EDTA, 0.1 M NaCl, 0.05 M Tris buffer (pH 7.2) and the DNA was extracted sequentially, twice with equal volumes of chloroform:isoamyl alcohol (24:1). After dialysis against 0.006 M Tris (pH 7.5) the contaminating RNA was eliminated by a treatment for 1 h at 60 °C with ribonuclease (Calbiochem) at 0.05 mg ml⁻¹ in 0.01 M NaCl, 0.0005 M EDTA, 0.002 M Tris (pH 8.0). The purified DNA was then dialysed exhaustively as above. No contaminating bacterial DNA was detected in whole-phage DNA preparations subjected to Agarose gel electrophoresis. Melting profiles also demonstrated the homogeneity of the DNA preparations, and showed that the mole fraction of guanine plus cytosine in both β -tsr3 and γ -tsr1 was 0.54. These data, together with microscopic evidence, show that corynebacteriophage DNA is double stranded.

To determine the molecular mass of the phage genomes, whole phage DNA was prepared and mounted on grids for examination in the electron microscope by the Kleinschmidt technique¹¹. RSF2124, a 7.4×10^6 molecular mass derivative of the *Escherichia coli* plasmid ColE1 with an inserted Tn3 (provided by P. Shipley) was used as a molecular mass standard¹². The contour lengths of 21 linear β -tsr3 molecules were measured and from these a mean molecular mass of

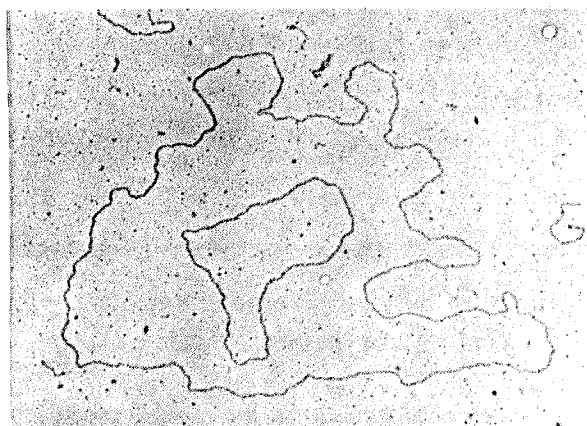


Fig. 1 Electron micrograph of γ -*tsr1* DNA spread for the determination of molecular mass. The small molecular mass standard, RSF2124, is encircled by γ -*tsr1* DNA.

$22.9 \pm 0.31 \times 10^6$ (\pm standard error) was calculated. Similarly, 20 γ -*tsr1* molecules had a mean molecular mass of $25.2 \pm 0.51 \times 10^6$. These data compare favourably to the estimate of 22×10^6 for wild-type β DNA reported by Wolfson and Dressler (quoted in ref. 13). Circular molecules noted in these preparations (Fig. 1) were also measured. The molecular mass as calculated from the contour length of 21 circular β -*tsr3* molecules was $22.9 \pm 0.08 \times 10^6$, and was $24.9 \pm 0.07 \times 10^6$ for 19 circular γ -*tsr1* molecules. The presence of circular molecules in the DNA preparations suggests that packaged β and γ phages have sticky, single-stranded ends similar to the lamboid phages of *E. coli*. Laird and Groman, in their study of the prophage map of β phage⁷, postulated that circularisation of the vegetative phage genome precedes integration into the bacterial genome. Our findings provide physical evidence that this step is possible.

Heteroduplexes of the two corynebacteriophage mutants

were prepared¹⁴ and examined in the electron microscope. Two typical heteroduplexes are shown in Fig. 2a. Three single-stranded regions of nonhomology were observed, two deletion-insertion loops (DI-1 and DI-2) and one substitution bubble (S). Preliminary calculations showed that the two single-stranded DI loops were most reasonably assigned to γ (see below), and therefore the total duplex length of the hybrid molecule plus the length of S was attributed to β . With the duplex length of β , the molecular mass of its DNA and a conversion factor of 2.08×10^8 per μm (ref. 15), the absolute sizes of various segments of DNA in the heteroduplex were readily calculated. The end of the heteroduplex nearest to the DI-1 loop was arbitrarily chosen as the starting point, and the distances derived are shown in Fig. 2b. Loops DI-1 and DI-2 were assigned to γ -*tsr1* because the sum of these loops, 2.0×10^6 , corresponds closely to the 2.3×10^6 difference in the molecular mass of the two bacteriophage DNAs.

The most significant finding in these heteroduplex studies is that over 99% of β DNA is homologous to γ DNA. Only the 0.11- μm S segment fails to hybridise. Conversely, if the sizes of DI-1, DI-2 and the estimated length of β are summed to give the approximate size of γ , it can be calculated that over 91% of γ DNA is homologous to corresponding regions of β DNA. The DI loops were assigned to γ in these calculations. Further evidence of this close relationship is provided by the electrophoretic banding patterns of restriction enzyme digests of the two phage DNAs. DNA from β -*tsr3* and γ -*tsr1* was cleaved with restriction enzymes and the resulting fragments were compared by electrophoresis on Agarose gels¹⁶. The gels show that β and γ have remarkably similar patterns, although in all digests γ DNA displayed one or more bands not found in β DNA (Fig. 3).

The heteroduplex of β and γ DNA establish unequivocally that there is a close evolutionary relationship between these two phages, a conclusion previously suggested by circumstantial genetic, serological and morphological data. The restriction enzyme analysis also strongly

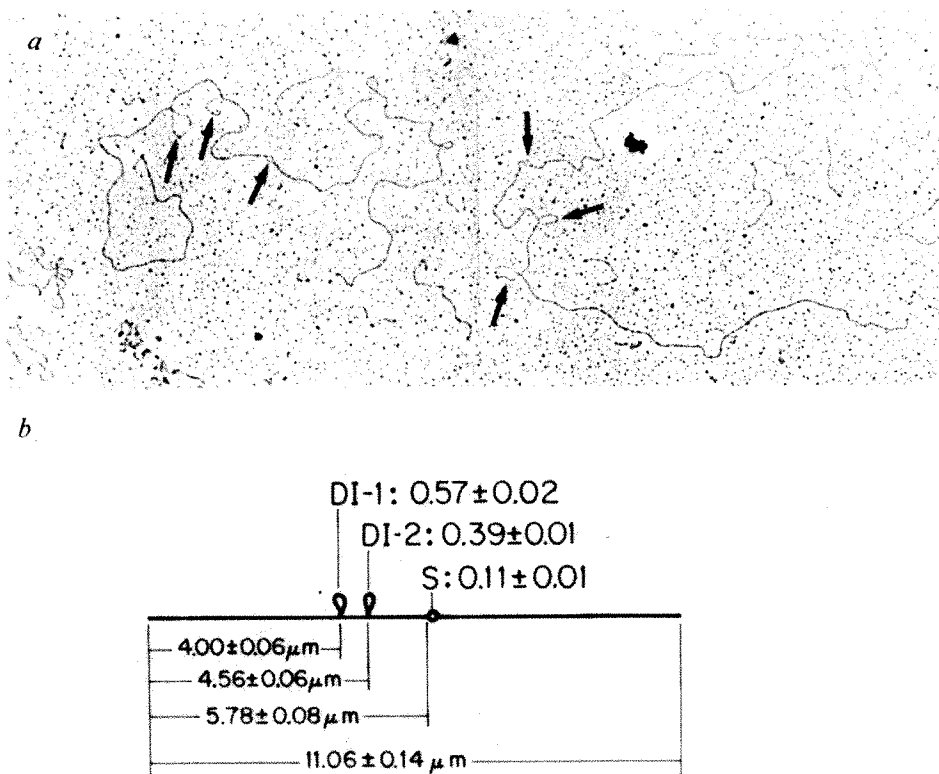


Fig. 2 a, Heteroduplexes between β -*tsr3* and γ -*tsr1*. Heteroduplexes were prepared by mixing 0.1 to 0.2 μg of each DNA in 0.25 ml of a 0.1 M NaOH, 0.01 M EDTA aqueous solution at pH 12.4–12.6 for 10 min, at which time the pH was reduced to 8.4–8.6 by adding 2.0 M Tris (pH 7.1). Formamide, 0.25 ml, was added and the mixture allowed to re-anneal at room temperature for 4–6 h at which time the DNA was spread and rotary shadowed as described¹⁴. All electron microscopy was done with a JEOL Model 100B electron microscope. b, Graphical representation of the heteroduplex.

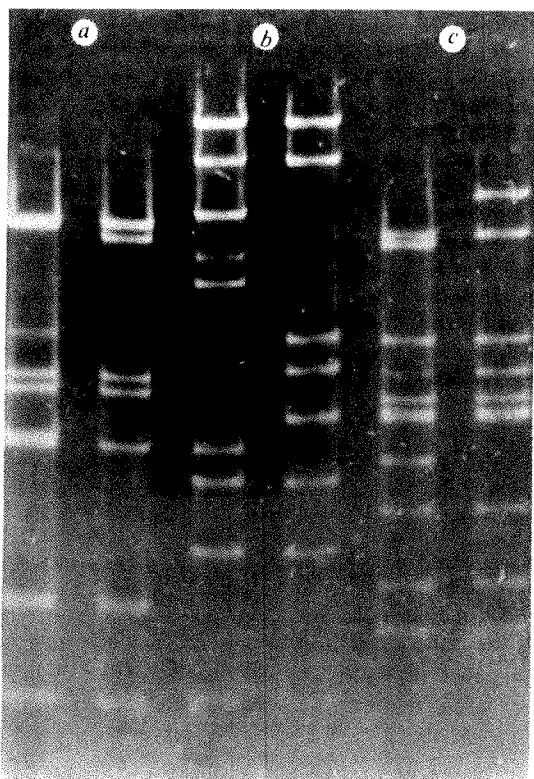


Fig. 3 Restriction enzyme digests after electrophoresis on 0.5% Agarose gels. *a*, *Sal*I; *b*, *BAM* H-1; *c*, *Eco* R1. In each case the γ -*tsr*1 DNA is in the left lane and the β -*tsr*3 DNA in the right lane. One minor band that appears at the bottom of the negative for the *Sal*I digest of both γ and β does not appear in this photograph.

supports this conclusion. The high degree of similarity seen in the DNA pattern of the digest is more significant than the differences, since small changes in nucleotide sequences (including single base changes) can alter DNA banding patterns. The presence of one or more minor bands in the Agarose gels (Fig. 3) indicates that there is a small amount of heterogeneity in the digests of γ DNA. Since this heterogeneity was not eliminated by clonal reisolation it is assumed to be intrinsic to the γ population. The reason for this heterogeneity is not clear, but the limited nature of this variation does not affect the primary conclusions of this paper.

Data from the restriction enzyme digests also confirm the molecular mass of β and γ DNA obtained by direct measurement. Standards were used to calculate the approximate masses of each fragment in the digest and these were summed to provide an independent estimate of the size of the two phage DNAs. In the three digests used in these calculations, β -*tsr*3 varied in size from 21 to 22×10^6 and γ -*tsr*1 varied from 22 to 23×10^6 . Although these figures are slightly smaller than those reported above from direct measurements, they are confirmatory, particularly since the summation of fragment weights is less precise than calculations from contour length. Significantly, in each enzyme digest γ DNA was consistently 1 to 2×10^6 molecular masses larger than β . This closely approximates the size differences calculated from the direct measurements.

On the basis of previous observations (see above), a large degree of homology was expected between β -*tsr*3 and γ -*tsr*1. However, it is of particular interest that though these phages are heteroimmune their DNAs are 99% homologous. This suggests that parental β and γ phages are either recent evolutionary derivatives, one from the other, or recently shared a common ancestor. If so, then heteroimmunity must have arisen either through a small number of muta-

tions or through recombination with a distantly related corynephage. Strong selective pressure for genes outside the immunity region would also account for the high degree of homology, but the extent of homology makes it less likely that this is the primary conserving mechanism.

It is not possible to identify the regions of nonhomology between β and γ phages without appropriate mutants. Because these phages are heteroimmune the immunity region is an obvious candidate for the region of nonhomology seen in the substitution bubble. Although β phage is *tox*⁺ and γ is *tox*, it is known that γ carries at least a portion of the *tox* gene⁸. It is possible that the cryptic nature of the *tox* gene in γ is the result of an insertion of nonhomologous DNA into the *tox* gene. If this were the case it would account for one or both of the loops.

We thank Mr Ron Gill for his advice on techniques and helpful discussions. This research is supported by USPHS grant AI-10492 and NSF grant 75-14174.

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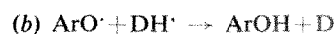
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CIDNP in tyrosyl protons of luliberin

We report here on the possibility of extending CIDNP (chemically induced nuclear magnetic polarisation)-assisted NMR studies of the tyrosyl unit to medium sized peptides. Our previous studies indicate that, strong ¹H nuclear polarisation may usually be obtained in phenols in the presence of dyes such as fluorescein or any of its halogenated derivatives, such as rose bengal, erythrosin B or eosin Y, and others excited to their triplet state¹⁻⁴. The polarisation is due to the H atom transfer from the hydroxyl of the phenol ArOH to the triplet excited dye ³D,



and back,



Steps (a) and (b) were shown to be chemically reversible, the nuclear polarisation resulting from the magnetic interactions in spin-correlated radical pairs ArO[•] + DH[•].

We recently reported that the phenolic amino acid tyrosine and some of its simple derivatives as well as small tyrosyl peptides show a similar phenol CIDNP in certain well defined conditions⁵ (Dr R. Kaptein (Groningen) has performed similar

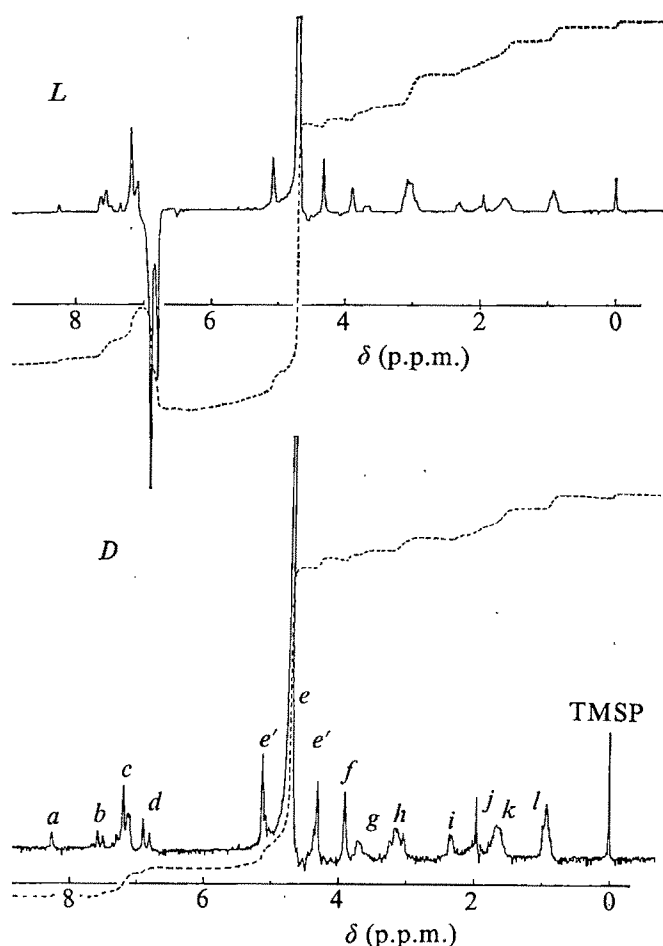


Fig. 1 (—) ^1H 90 MHz FT NMR spectra of luliberin acetate (0.01 M) in D_2O in the presence of fluorescein (2×10^{-3} M). Broken lines (---) give the integration curves. Each spectrum is the result of 256 accumulated pulses. *D*, dark spectrum; *L*, light spectrum (RF pulse preceded by 1 s ultraviolet irradiation period). The protons are assigned according to Vessel *et al.*⁸ *a-d*, aromatic rings: *a*, His; *b*, Trp; *c*, Trp and Tyr meta to OH; *d*, Tyr ortho to OH; *e* and *e'*, HDO impurity; *f*, methylene of Gly-NH₂; *g*, Ser methylene; *h*, Tyr, His, Trp and Arg methylene; *i*, Glu and Pro methylenes; *j*, acetate; *k*, Leu and Arg methylenes; *l*, Leu methyl; TMSP, trimethyl silyl propanoic acid, d_4 used as $\delta = 0$ standard.

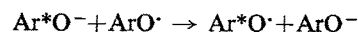
tyrosine CIDNP studies with other unspecified dyes). In our studies, carried out in D_2O solutions in the presence of fluorescein, strong CIDNP effects were obtained on the tyrosine ring protons ortho to the OH group and on the tyrosine methylene protons.

The particular substrate chosen in this investigation of the triplet dye-induced tyrosyl CIDNP in peptides was luliberin^{6,7}, the hypothalamic luteinising hormone releasing factor (LRF). This linear decapeptide of amino acid sequence pGlu-His-Trp-Ser-Tyr-Gly-Leu-Arg-Pro-Gly-NH₂, was used as the acetate after repeated exchange of its acidic protons with D_2O .

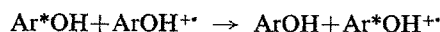
Figure 1 (*L*) shows the effect of ultraviolet irradiation on the proton NMR spectrum of a 0.01 M solution of luliberin in D_2O in the presence of fluorescein (0.002 M), which acts as the H atom photoabstractor. The tyrosyl ring protons ortho to the hydroxyl group (*d*) show a 17-fold negative enhancement *E*. Signal *h*, due to the tyrosyl methylene protons and to methylene protons of His, Trp and Arg⁸ similarly shows a 2-fold enhancement (*A*). The signs of the CIDNP effect for ortho and methylene protons (— and +) are the same as those found in the other phenols¹⁻³, in complete agreement with the analysis

based on the radical-pair model of CIDNP⁹. The *E* and *A* effects in luliberin are similar in magnitude to the effects observed in other tyrosine compounds with the exception of H-Tyr-Tyr-OH, where considerably larger effects were observed (ref. 5 and K. A. M. & C. G., in preparation). The two synthetic derivatives¹⁰ [*L*-Ala⁶]LRF and [*D*-Ala⁶]LRF were also studied, and gave almost identical results.

Preliminary results in smaller peptides indicate that tyrosine ^1H CIDNP is strongly pH dependent. This effect should probably be attributed to the nuclear relaxation of the polarised nuclei catalysed by fast degenerate electron exchange processes (* denoting nuclear polarisation)



and



The efficiency of these is due to the short T_1 relaxation times of the paramagnetic species $\text{Ar}^*\text{O}^\bullet$ and $\text{Ar}^*\text{OH}^{+\bullet}$.

Further experiments are being carried out to determine the scope of the method and the role of factors such as structure of peptide and of photoabstractor, and tyrosyl unit accessibility.

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Received 22 September; accepted 16 December 1977.

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matters arising

Upwelling by icebergs

NESHYBA¹ argues for the importance of iceberg melting below the thermocline in introducing nutrient rich water into the surface layer. I believe she overrates this effect. She assumes that icebergs melt as a single piece and does not even consider calving as part of the deterioration process. Icebergs that are protected from wave action by sea ice are observed to deteriorate little. Icebergs begin to undergo rapid deterioration when they emerge from the sea ice into the open sea². Wave action progressively melts a groove at the waterline by turbulent mixing. I have observed these grooves to have a depth of up to 7–10 m. The sides of the iceberg then fail, producing a large number of small icebergs and 'growlers' and much fine brash. These small icebergs and growlers, which in most cases would be less than 30 m long and have a maximum draft of 20–25 m, are subject to the same erosional forces. Although no hard figures exist at this time, I would estimate that more than 80% of the melting of an iceberg takes place in a surface layer no deeper than 20 m which is in most cases shallower than the main thermocline.

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Cadmium in seabirds

I FEEL that the article on cadmium in marine animals by Bull *et al.*¹ contains a number of misstatements. Seabirds do not generally contain higher residues than marine invertebrates; as long ago as 1956 Mullin and Riley² reported that molluscs may contain up to 500 mg of cadmium per kg in their digestive organs and renal glands, a level not yet equalled by birds, and Holdgate³ reported much the same levels in healthy guillemots *Uria aalge* from British waters as Bull *et al.*¹ do for other marine animals.

Anderlini *et al.*⁴ only investigated six, not seven, bird species, and then only looked at the eggs, which contain little cadmium, for three of them; they included birds from the Atlantic as well as the Pacific and Antarctic. In the three

species where they tested the livers, the most novel finding was perhaps not so much the presence of a mean level of 53 mg of cadmium per kg in ashy petrels *Oceanodroma homochroa* from the polluted coast of California, as that of nearly half as much (means of 20–28 mg per kg) in the livers of snow petrels *Pagodroma nivea* resident in the Antarctic pack ice and Wilson's petrels *Oceanites oceanicus* breeding in Antarctica and migrating into different northern oceans. Similarly, the main point of interest about the puffins *Fratercula arctica* examined by Parslow *et al.*⁵, only one of which was taken alive at a colony as reported by Bull *et al.*¹, was that the two healthy birds from remote west coast colonies contained much more cadmium than the six mainly found washed up on beaches from the east coast. They also reported surprising levels in a herring gull *Larus argentatus* (12 mg per kg) and a Manx shearwater *Puffinus puffinus* (24 mg per kg) as well as the fulmar *Fulmarus glacialis* liver level of 159 mg per kg actually quoted by Bull *et al.*¹ which is higher than any reported by the latter from the same laboratory when they claimed 'considerably higher levels than any previously found'; the ashy petrel mean is in fact also near their highest liver level.

In addition to their references to past evidence for the normal occurrence of high cadmium levels in pelagic seabirds, Bull *et al.*¹ also seem a little careless in their discussion of their own observations on the occurrence of cadmium in sea-skaters *Halobates micans*. They state 'some of the areas where the higher cadmium residues occur, for example off the coast of West Africa,

have little industrial activity, but have upwelling currents of cold water which bring to the surface nutrients, and possibly cadmium too. Areas of ocean upwelling are known to be important wintering locations for pelagic seabirds.' Leaving aside the question whether the bird species quoted do in fact winter in upwelling areas, the map published by Bull *et al.*¹ shows no particular concentration of cadmium in *Halobates* in such areas, but in fact an apparently random distribution of levels above 25 mg per kg throughout the area sampled including the centre of the Sargasso Sea, which is more celebrated for sinking water than upwelling.

Bull *et al.*¹ attribute to me the statement that individual birds are becoming increasingly contaminated with cadmium through feeding in areas of local pollution around the British coast. In point of fact, I ended the paragraph which I devoted to cadmium⁶ 'it would appear that like some other marine animals pelagic seabirds may accumulate exceptional amounts of this element, whose effects are still obscure'. This statement attributed to me comes from the next paragraph summarising observations of heavy metals in general, though as Holdgate³ reported up to 13 mg per kg cadmium in dead guillemots washed up in western Britain in the autumn of 1969 when the level in normal birds was low, it seems possible that there may be some local pollution with cadmium as well as other metals of birds frequenting our offshore waters. If this is the case with either cadmium or any other pollutant it seems doubtful whether it will be detected by looking at birds from remote breeding stations which feed elsewhere, rather than at individuals dying on the spot. It would also be helpful if Monks Wood Experimental Station could place all their other estimations of pollutants on record.

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Matters Arising

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Transmitter release statistics are meaningful

BARTON and Cohen¹ recently attempted to show that statistical analyses of neurotransmitter release, especially those based on a simple binomial model, are of dubious validity. Given their point of view, the authors might have asked: are any statistics meaningful? Every statistical analysis is based on certain assumptions, some purely formalistic, some containing empirical elements. If the statistician assumes an underlying binomial distribution then he tacitly assumes that there are a number of units, each of which may act independently in two ways with a certain probability for each way, the respective values for these probabilities being the same for all units. A binomial assumption was first satisfactorily applied to transmitter release at the crayfish neuromuscular junction². In general terms such an analysis assumes units involving a certain class, C , of probability distributions. The basic parameters of this class are (1) the form of dependence of the units; (2) the number of units, n ; (3) the probabilities of release, p_1, p_2, \dots, p_n . The binomial is the simplest subclass of the whole with independence, n constant and $p_1 = p_2 = \dots = p_n$. Each element of this subclass can be approximated in many ways by more complicated elements of C , but is there any value in that when the simplest analysis suffices?

For example, in the case of falling bodies a random exponent, distributed about 2.00001, would serve as well as the classic quadratic relation; orbits other than a simple ellipse could suffice to describe the path of the earth around the sun.

These examples illustrate the 'principle of parsimony' which postulates that one's observations be described by as simple a model as possible. Experimental observations which cannot be described by the simplest model can then be used for gaining insight into the underlying processes in the real world and are described by, stepwise, more complex models (for example, the branch-block model proposed to explain experimental findings at the frog neuromuscular junction³).

The computed example of Barton and Cohen presents the result of a concatenation of binomial trials with a wide range of p values. However, it is difficult to conceive that over 100 quanta with such low release probabilities ($p < 0.01$ in over 70% of quanta) would result in so few failures of release (their Fig. 1b shows only 2–3 failures). Nonetheless, in the result as presented, the simulated and predicted curves would seem to be different; indeed, the χ^2 value quoted indicates that, had they simulated about 250 trials instead of 100, a 5% level of significance would have been achieved with the same relative differences between

the two histograms. This emphasises the importance of an adequate number of samples for satisfactory analysis of transmitter release.

We find the paper of Barton and Cohen a negative one, which makes no contribution to the real need, of which our own experimental results have made us particularly aware. This need is for a comprehensive theoretical treatment of particular generalisations of the binomial model, of which the branch-block model is simply one. Only then will interpretation of experimental results be soundly based; but without the experiments science can become merely a meaningless play with models.

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Kraepelinstraße 2, FRG

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BARTON AND COHEN REPLY—We indicated¹ that many different p distributions can lead to histograms of the quantal content which are indistinguishable from the binomial predictions. There are a number of reasons for rejecting the plea of Hansert, Wernig and Carmody that only the 'simplest analysis' should be considered. As we mentioned in our letter, the binomial assumption conflicts with at least two experimental observations. First, quantal contents greater than the binomial estimate of n have been recorded at the crayfish neuromuscular junction; this is equivalent to a release of more than the total number of available quanta. Second, the timing of spontaneously released quanta is clustered, implying temporal or spatial non-uniformity of the release process. It is not obvious how the branch-block modification can explain this property of spontaneous release.

The implications of statistical analyses of quantal release are critically dependent

on the assumed probability distribution. n is generally assumed to represent an extensive property of the release process and p an intensive one. However, if the binomial assumption is false then a change in the p distribution of a fixed number of quanta would be interpreted as a change in both n and p , or even as a change in n alone.

The principle of parsimony is used to select the simplest of a set of equivalent hypotheses². However, this principle is irrelevant because the predictions of the assumptions in question are different and experimental distinction of these hypotheses is possible.

We would like to clarify three other points raised by Hansert *et al.* First, there are only two failures of release when 70% of the quanta have $p < 0.01$ because those quanta with high p values effectively prevent large numbers of failures. If a single quantum had $p = 1$ then no failures would ever occur. Second, we described a simulation of 100 trials—a sample size often used by some workers³—but simulations of 1,000 trials produced similar results (see Table 1). It is incorrect to assume, as Hansert *et al.* did, that the relative shape of the quantal-content histogram remains the same when the sample size is increased. After publication of our letter it was brought to our attention that Brown, Perkel and Feldman⁴ had performed a similar analysis in 1976 and had reached the same conclusions that we did; they used 1,000 trials and studied the effects of temporal and spatial variations in n or p . The distributions of quantal contents remained indistinguishable from the binomial predictions even at this high sample size. Third, we agree that a general theoretical treatment would be valuable, but the successful development of this seems unlikely without more data.

These arguments suggest to us that the assumption of binomial release is unlikely to be valid, despite its mathematical simplicity. We believe that the contribution of our letter can be determined only by its success or failure in stimulating new experiments designed to differentiate between the various hypotheses.

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Table 1 The results of six simulations

χ^2	Degrees of freedom	P
5.421	7	0.61
0.924	5	0.97
6.921	6	0.34
3.094	8	0.93
8.172	6	0.23
9.305	6	0.16

The distribution of the release probability of the 100 quanta was generated in the same way as that in Fig. 1a of our earlier letter¹ ($\bar{z} = -5$, $\sigma_z^2 = 2$). 1,000 trials were performed in each simulation. The χ^2 value compares the simulated distribution of quantal contents with the best-fitting binomial distribution. The P values give the probability that a value of χ^2 greater than the observed value can arise by chance.

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reviews

Phylogeny of immunity

Nicholas Cohen

Immunity in Evolution. By John J. Marchalonis. Pp.xx+316. (Edward Arnold: London, 1977.) £10.

RECENT years have witnessed a surge of quality research and heightened theoretical interest in the plethora of problems posed by the phylogeny of immunity. Within the past two years, this activity has led to the birth of the journal, *Developmental and Comparative Immunology*, and to the formation of the Division of Comparative Immunology within the construct of the American Society of Zoologists. This activity has also been translated into several reviews, publication of the proceedings of four substantive international symposia, and, as well might be expected, the appearance of three monographs. Dr Marchalonis' labour of love, *Immunity in Evolution*, is the most recent single-authored volume to appear. Fortunately, for readers and authors (and publishing companies), redundancy has been minimised, since each monograph vividly mirrors its author's unique interest, expertise, and personality.

Marchalonis devotes a full 10 chapters to a discussion of the evolution of immunoglobulin structure and function, an area in which his first-hand experience and insight is well-known. Following a detailed review of the structure of mammalian immunoglobulins and the arrangement of immunoglobulin genes in mammals, Marchalonis digests, in a well organised and well written fashion (and in separate chapters) the large body of literature dealing with invertebrate humoral mediators of defense; the phylogenetic emergence of IgM; light chains; the distribution of immunoglobulins distinct from the IgM class; variable regions; antigenic studies of immunoglobulins of eutherian mammals; molecules that might possibly be related to immunoglobulins; and the origins of antibody diversity. His text is more than a paraphrasing of the original papers; it is an assimilation that focuses on concepts and enigmatic problems as well as on facts.

Although Marchalonis does not deal with the evolution of the cellular basis of immunity with the same detail and

perceptive depth that he devotes to humoral immunity, he does critically discuss many of the salient aspects of quasi-immune recognition and effector systems of invertebrates; the emergence of lymphoid cells and organs; the phylogenetic distribution of cell-mediated immune functions; memory and tolerance; and manipulations of the immune systems of ectothermic vertebrates with temperature. It is in these chapters, however, that one shortcoming of the book emerges, a blemish that is revealed only because of the very vitality of the field with which it deals. Although fewer than 2% of the more than 700 cited references were published in 1975 (none were later), it is precisely during the past three years that many of the queries commendably raised by Marchalonis that bear, for example, on the evolution of lymphocyte heterogeneity, the evolution of the major histocompatibility complex, cell cooperation, and the selective effects of temperature on a subset of T cells, have been approached and partly answered. It is also during this period that major concepts in cellular immunology have emerged that may have striking ramifications in understanding the evolution of immunity

(for example, associated recognition). In short, the time from submission and revision of the manuscript to its publication this year was too long.

Fortunately, it is relatively easy for the interested reader to remedy this understandable problem. He or she need only peruse one of the recent photo-offset published proceedings of the symposia held in 1975-77 (*Developmental Immunology*, *Am. Zool.*, **15**, 3-213; 1975; *Immunologic Phylogeny*, ed. W. H. Hildemann and A. A. Benedict, *Adv. exp. Med. Biol.*, **64**, Plenum: New York; 1975; *Phylogeny of Thymus and Bone-Marrow-Bursa Cells*, ed. R. K. Wright and E. L. Cooper, North-Holland: Amsterdam; 1976; *Developmental Immunobiology*, ed. B. M. Solomon and J. D. Horton, North-Holland: Amsterdam; in press) to update their information. Marchalonis' book has provided many of the provocative questions and has predicted several experimental scenarios; these symposia provide at least some of the answers. □

Nicholas Cohen is Associate Professor of Immunology at the University of Rochester School of Medicine and Dentistry, Rochester, New York.

Liquid semiconductors

Liquid Semiconductors. By Melvin Cutler. Pp. 226. (Academic: New York and London, 1977.) \$19.50; £13.85.

PHYSICISTS weaned on the nearly-free-electron theory of solids find it satisfying, if at first surprising, that the properties of most liquid metals can be treated within this approximation. As long as the scattering of the conduction electrons by the non-crystalline arrangement of atoms is weak, perturbation theory can be used, as for example in Ziman's theory of electrical conductivity. It seems that many of the recently discovered metallic glasses can also be described in these terms. However, if the scattering is so strong that the mean free path becomes comparable to the average lattice spacing, then perturbation theory is no longer adequate and new approaches must be sought. This is the situation in certain liquid

metals and in all amorphous and liquid semiconductors.

Concepts required for an understanding of the physics of amorphous and liquid semiconductors have evolved over the past ten years. It is clear that many properties are determined by the short range or local configuration of atoms—that is, bond length, bond angle and coordination number. These determine the main features of the distribution in energy of the electronic states. It is quite proper, therefore, that the author of this book should stress the tight-binding or molecular-bond model when interpreting experimental behaviour. Electrical conductivity, Hall effect and other transport properties demand special treatment, since the concept of well defined wave vectors for the electrons becomes inappropriate and the relaxation time approximation breaks down. The author describes the

current level of understanding of these properties, calling for the most part on models developed to describe amorphous semiconductors. For liquids, of course, further difficulties arise. The local atomic configuration and hence the electronic structure changes not only with time but also with temperature. Thus, if measurements are made as a function of temperature, one has to deal with a different material at each temperature.

The field is not short of experimental results and these are presented in several chapters, one devoted to physical properties, one to physico-chemical properties and metallurgical properties, and two others to specific alloys. The book is valuable for this collection alone. It is not surprising to find Ti-Te alloys discussed extensively, since this system has not only been exhaustively investigated by the author himself, but also exhibits so many of the diverse and interesting properties characteristic of liquid semiconductors. Experimentalists will appreciate the short chapter on techniques.

It behoves a reviewer to draw attention to faults lest it be thought that he is a friend of the author (which he is) or else in league with the publishers (which he is not). My only criticism (apart from an unfortunate interchange of the words band and bond between two section headings in the list of contents), is that tantalising extracts of theory and models, described in detail in later chapters, are interspersed with experimental data presented earlier in the book; yet if the reader tries the theoretical chapters first, he is confronted with tantalising extracts of data. One has to learn, however, to find one's way around any book of this kind and specialists in the field will certainly have no trouble.

The price came as a pleasant surprise (by today's standards) and the book *has* to be good value for such an admirable condensation of a difficult subject by a leader in the field.

E. A. Davis

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Group theory or general relativity

Group Theory and General Relativity. By Moshe Carmeli. Pp. 391. (McGraw-Hill: New York and Toronto, 1977.) £16.85.

THERE is no doubt that a textbook which links together the topics of group theory and general relativity is very welcome. The jacket of this book proclaims that it is the first book on "the applications of group theory to general relativity", but I have some reservations about whether this is an appropriate description. Reading the book gives the impression of two research monographs grafted together: one on group theory, the other general relativity. This does not mean that the contents are not useful, but some stronger motivation for the conjunction of topics would have been desirable.

Field theoreticians are well used to relying heavily on group theoretical techniques, particularly with the recent activity in the area of gauge theories. When it comes to general relativity, group techniques are less powerful because of the absence in general of metric-preserving global diffeomorphisms. Of course, interest centres on special model spacetimes with particular symmetries, and group theory has long played an elegant and useful role in elucidating and classifying their properties. More general classes of

spacetimes, however, can be fruitfully investigated using group methods. Especially important are those that admit asymptotic symmetry groups, and the formulation of concepts such as asymptotic energy momentum, and multipole moments of the gravitational field for isolated bodies relies heavily on the analysis of these groups. In addition to this, much attention has recently been directed to gauge theories in curved spacetime, in which the gauge group continues to play an important part.

This book covers much of the material relevant to these applications, but in a somewhat shapeless form. Readers who have no previous knowledge of one or the other (or both) central topics may find the presentation a little stark with essential facts simply run off. I would recommend some previous background reading, at least for the group theory, from the books of Naimark or Gel'fand *et al.* These are recommended by the author in his introduction, and Carmeli's treatment is closely similar to these.

The first six chapters contain little general relativity, dealing extensively with the representations of the rotation and Lorentz groups. Chapter 3 treats the spinor representations of the Lorentz group. Chapters 4, 5 and 6 tackle in great depth the principal, complementary and complete series of representations of $SL(2, \mathbb{C})$.

The author's discussion of relativity begins with chapter 7. The treatment is standard and traditional, and no attempt seems to have been made to

present the material in any especially novel or enlightening way, or to exploit many of the techniques of modern differential geometry. It is here that one most feels the need for stronger links with the group theory and motivating remarks. The author goes on to develop a detailed discussion of the spinor formulation of general relativity, and it is particularly useful that this approach, advocated by Roger Penrose, and for so long in widespread use among relativists, is dealt with in an integrated fashion in a student textbook. It is frustrating, though, that many useful results of spinor calculus are relegated to the exercises.

In chapter 9, Carmeli takes up the subject of gauge theories, and after a preliminary discussion of electromagnetic and Yang-Mills fields attempts to formulate general relativity also as a gauge theory. The Newman-Penrose formalism is obtained from a gauge theory approach, though perhaps its physical relevance could have been better described. The Goldberg-Sachs theorem is proved in chapter 10, but its importance is not made transparent.

The final two chapters of the book deal with solutions of the field equations of general relativity and the Bondi-Metzner-Sachs group. There are also several appendices. Once again, all the details are competently collected together, but the uninitiated reader may have difficulty in understanding their wider importance or relevance. Perhaps these people would do well to consult the final paragraph of the Introduction which contains a quotation of Abdus Salam, who learned group theory from Racah: "After attending these lectures I thought this is really too hard; I cannot learn this; one is hardly ever likely to need all this complicated matter. I was completely wrong".

In spite of the specific criticisms concerning the presentation, I find this book a timely addition to the many research papers on these two most important topics. It should prove useful as a teaching text and, at least in conjunction with some background material, it could be used as a course book for either group theory for physicists, or more specifically for relativity courses. The style is straightforward and information retrieval easy. Many exercises are given with each chapter and an extensive bibliography is added. It is elegantly produced, and at £16.85 is not over-expensive for a mathematical book of this length.

Paul Davies

Paul Davies is Lecturer in Mathematics at King's College, University of London, UK.



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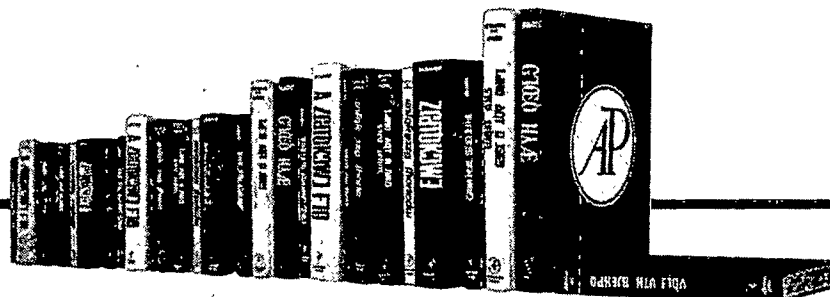
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Coastal zone ecology

The Coastline. Edited by R. S. K. Barnes. Pp. 356. (Wiley: Sussex, New York and Sydney, 1977.) £12.50; \$24.50.

By a nice piece of timing, the publication date of this book coincided with the First European Ecological Symposium on Ecological Processes in Coastal Environments at Norwich, where it was exhibited by the publishers. It certainly caught the attention of the conference participants who seemed to like what they saw; so did I. Having now had the opportunity to read it in some detail, my opinion has not changed.

The stated purpose of the book is to contribute to our understanding of the coastline's ecology and physiography in relation to land-use, management and the pressures to which it is subject. To meet this objective, the editor has brought together a team of contributors who have done an admirable job in condensing a relatively large amount of information into one very readable volume. After an introductory chapter on problems and pressures, there follow thirteen chapters, each concerned with one fairly uniform group of habitats. Seven chapters describe predominantly aquatic environments (lagoons, estuaries and foreshores) and six cover terrestrial environments (reclaimed land, cliffs, dunes and shingle). A common structure has been adopted for each chapter: a summary of the ecological and geomorphological processes, characteristic features and pressures, recommendations for methods of study, particular uses, and finally suggestions for conservation and management policies. Coastline management is dealt with in more detail in a concluding chapter.

Many of the examples given are of British coastal areas, but others are from mainland Europe and some from as far afield as Australia. The diagrams which illustrate the various sections are generally good but the reproduction of the (fortunately few) photographs is very poor. There are errors, but those I found are mostly minor spelling mistakes. However, inaccuracies do occur: for example, on p144, it is erroneously stated that the Nature Conservancy Council do not own land. I consider that there are also some errors of omission. Current proposals for energy generation from a Severn Barrage or wave energy converters are not included; the effect of either on the coast would probably be considerable.

Although thermal pollution is mentioned, the possible damaging effects on plankton by its entrainment in cooling water systems is not discussed. The introduction of alien species, which has already led to a great many changes in some ecosystems, is dealt with rather superficially. It would have been valuable if some developing ideas about various ecosystems were included, such as the probable part played by diatoms and some invertebrates in stabilising sediments by their mucus secretions. It is surprising how little is said about ecological and conservation evaluation of coastal ecosystems, surely an essential step in formulating any management proposals. Perhaps more would have been included on this subject had *A Nature Conservation Review* (for review, see *Nature*, 269, 271, 1977) been published a few years earlier.

Atmospheric highlights

Earth's Aura. By Louise B. Young. Pp. 305. (Alfred A. Knopf: New York, 1977.) \$12.95.

This is a book about some of the highlights of the history of atmospheric exploration, about the effect of man's activities on the atmosphere and about the effect of climate on man. In the first chapter we are taken up with pioneering balloonists rising to unbelievable heights in flimsy baskets as the main structures of the various regions of the atmosphere are introduced. The second and third chapters introduce us to clouds and cloud-seeding experiments, and describe thunderstorms by recounting the drama of Franklin's classic kite experiment and the experience of a German glider pilot caught in a cumulonimbus. The delicate balances which control some of the atmosphere's chemistry (including the ozone problem) are introduced in the next three chapters. The enormous variety of winds, jet streams and waves come next; then optical phenomena, the solar wind and the atmospheres of the planets are dealt with in the same dramatic style. Modern speculations about the way the climate may change are discussed in a final chapter.

It is a fascinating account written in an enthusiastic, dramatic, sometimes gripping, style. The material has been effectively selected so that the book is not only very readable but also informative. Some beautiful colour pictures from space are included in the collection of ten illustrations in the centre of the volume.

Both the general reader and the

As stated in the concluding chapter, we still have to rely heavily on a descriptive approach to many ecosystems, as not enough is known to quantify them; we still have some way to go before we can be confident in our predictive capabilities as coastal managers. In relation to this, it would have been valuable to have had a discussion of the last chapter which concerned itself more fully with an appraisal of the state of the coastline. Nonetheless, I strongly recommend the book to those concerned with coastal management as well as to those seeking a lead into the study of this fascinating zone.

R. Mitchell

R. Mitchell is a Marine Biologist and the Chief Scientist's Team at the Nature Conservancy Council, Huntingdon.

specialist will be left with a new perspective on the environment and a new enthusiasm for the continued quest. "Weather is probably the most dominant factor in determining the quality of human existence", we are reminded, "but although we have set foot on the Moon, have identified organic molecules in the tenuous dust of outer space and measured the stars of galaxies a billion light years away, we still do not understand the intricate interplay of forces that make the earth's weather".

On the whole, the book does give the present state of knowledge in an accurate way. The emphasis is on two of the chapters, however, to leave the reader with a milder impression. One might have wished for instance that alongside the detailed description of the 1952 drought and the far-reaching changes brought about by the 1957 Clean Air Act, more had been described. Less attention is also given to the relatively small area of the influence of climate of sunspot cycles on the earth, whereas virtually no mention is made of the ocean/atmosphere interaction and feedback processes in the Arctic or Antarctic ice regions, the probably of much greater importance.

The cover describes this as a popular science writing at its best. It is a pleasure and discovery of the new problems that still face us. I have enjoyed reading it: it must be one of the best popular science books I have read deals with the atmosphere.

Further, for a well printed volume of 700 pages and some colour illustrations, the book is moderately priced.

J. T. Houghton

J. T. Houghton is Professor of Atmospheric Physics at the Clarendon Laboratory, Oxford, UK.

Recent scientific and technical books

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- BARKER, A. N., WOLF, J., ELLAR, D. J., DRING, G. J., and GOULD, G. W. (edited by). *Spore Research 1976*. Vol. 1: Pp.xvi+1-420. ISBN-0-12-078701-6. £14; \$27.35. Vol. 2: Pp.xvi+421-915. ISBN-0-12-078702-4. £16; \$31.25. (London and New York: Academic Press, a Subsidiary of Harcourt Brace Jovanovich, Publishers, 1977.)
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- BERKALOFF, André, BOURGUET, Jacques, FAVARD, Pierre, et LACROIX, Jean-Claude. *Biologie et Physiologie Cellulaires. 1. Membrane Plasmique, etc. Nouvelle édition entièrement refondue et augmentée*. (Collection Méthodes.) Pp.271. ISBN-2-7056-5876-9. (Paris: Hermann, 1977.)
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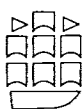
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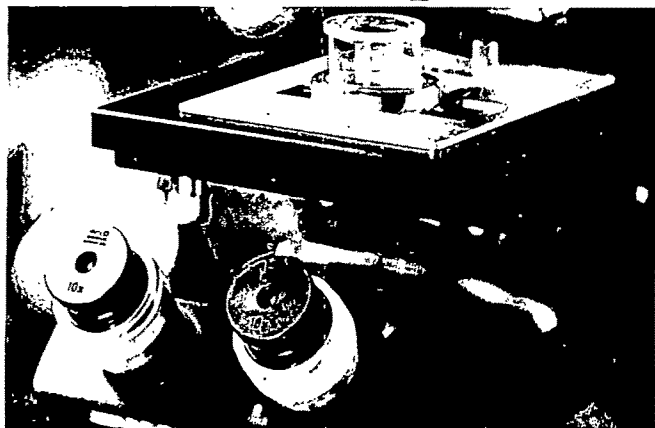
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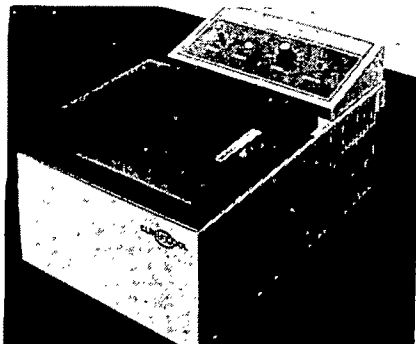
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- COLUCCI, Joseph M., and GALLOPOULOS, Nicholas E. (edited by). *Future Automotive Fuels: Prospects, Performance, Perspective*. Pp.ix + 380. ISBN-0-306-31017-1. (New York and London: Plenum Press, 1977.) \$47.40.
- ELECTRIC CIRCUITS: a First Course in Circuit Analysis for Electrical Engineers. By Members of the Staff of the Department of Electrical Engineering, MIT. (Principles of Electrical Engineering Series.) Pp.xxxiii + 782. (Cambridge, Mass.: The MIT Press, 1977.) \$6.95.
- FOSTER, Arthur R., and WRIGHTS, Jr., Robert L. *Basic Nuclear Engineering*. Third edition. Pp.xii + 548. ISBN-0-205-05697-0. (Boston, Mass. and London: Allyn and Bacon, Inc., 1977.) £9.75.
- HEARN, E. J. *Mechanics of Materials: An Introduction to the Mechanics of Elastic and Plastic Deformation of Solids and Structural Components*. Vol. 1: Pp.xxix + 1-394. ISBN-0-08-018749-8. £10. Vol. 2: Pp.xxii + 395-643. ISBN-0-08-020617-4. £7.50. (Pergamon International Library of Science, Technology, Engineering and Social Studies International Series in Materials Science and Technology, Vol. 19.) (Oxford and New York: Pergamon Press, 1977.)

what's new – centrifuges

These notes, prepared from material provided by the manufacturers, are intended to give an outline of the range of products on the market. More detailed information may be obtained by circling the appropriate numbers on the Reader Enquiry Card, bound inside the cover. A feature on balances will appear in the 8 May issue.

Damon/IEC. The IEC DPR-6000 high-capacity mid-speed refrigerated centrifuge features a digital speed readout that gives precision to 10 r.p.m., eliminating the need for periodic speed calibration. The DUR-6000 is fitted with a flexible drive shaft, a brush-wear indicator, durable range timer, variable electric brake and precise temperature control. The DPR-6000 attains a maximum speed of 6,000 r.p.m. and maximum force of 7,275g. Maximum capacity is 6 l. The IEC range also includes eight models of bench-top centrifuge and the B-20A and B-60 ultracentrifuges.

Circle No. 51 on Reader Enquiry Card.



The IEC Clini-cool refrigerated bench-top centrifuge

Gallenkamp. The Gallenkamp CFC-301 bench-top centrifuge has a maximum total capacity of 200 ml and has a top speed of 5,000 r.p.m. A built-in timer provides automatic control of running time for up to 20 min, if required. The self-balancing action of the motor ensures that centrifuge tubes need only be balanced by eye. Speed control is provided and choice of swing-out and angle heads is available. Centrifuge CFD-400 is suitable for a large number of routine and research applications, and has a maximum speed of 5,000 r.p.m. and total capacity up to 1,000 ml.

Circle No. 52 on Reader Enquiry Card.

DuPont Instruments (Sorvall). The GLC-3 bench-top model has a capacity of 80 tubes from 10×75- to 12×75-mm at speeds of up to 3,400 r.p.m. and forces of

1,240g. The GLC-2B also takes 80 tubes, providing speeds up to 2,600 r.p.m. and 1,240g. Higher g forces are attained by using buckets; the GLC-2B providing speeds up to 6,000 r.p.m. with fixed-angle rotors. The Sorvall RC-5B superspeed refrigerated centrifuge is equipped with a built-in automatic rate controller for soft starts and stops. Temperature, speed and time are programmed automatically. The rate of acceleration can be varied between start and 100 r.p.m. to give optimum acceleration for density gradient runs with vertical and zonal rotors. The Sorvall OTD-50 ultracentrifuge provides speeds up to 50,000 r.p.m. and forces up to 300,000g and the OTD-65 provides 65,000 r.p.m. and 429,297g. Both ultracentrifuges are self-contained: the self-contained cooling unit eliminates the need for external plumbing, filter and valves. The RC-5 superspeed and the OTD ultracentrifuges can be fitted with Sorvall vertical rotors which allow the rapid performance of density gradient separations.

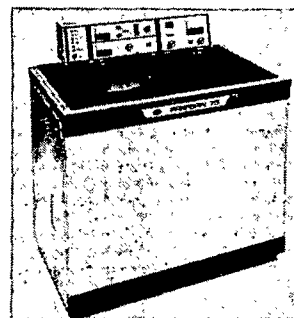
Circle No. 53 on Reader Enquiry Card.

Beckman. The TJ-6 bench-top centrifuge is available with or without refrigeration. The TJ-6 provides speeds of up to 2,700 r.p.m. and 1,520g. Fixed-angle rotors (10×50 ml and 24×15 ml) are rated for 5,700 r.p.m. and 4,470g. The Microfuge B miniature centrifuge holds 48 250- or 400-μl tubes or 18 1.5 ml tapered-bottom tubes. It accelerates almost instantaneously to top speed and can spin down blood cells or protein precipitates in 1 min. Separations for RIA take 3 min. The Model J-21C floor-model centrifuge is designed as a general laboratory centrifuge for initial processing of large particles in large volumes at up to 21,000 r.p.m. and 50,330g. The Beckman range of preparative ultracentrifuges includes the Model L5-40, with a maximum speed of 40,000 r.p.m., 284,000g and the L5-75 which can operate at 75,000 r.p.m., 504,000g. The Beckman Airfuge is a miniature table-top ultracentrifuge capable of rapidly separating small volumes. The rotor holds six 175-μl samples and attains 100,000 r.p.m., 160,000g in 30 s. The Airfuge is powered by standard laboratory air pressure.

Circle No. 54 on Reader Enquiry Card.

MSE Scientific Instruments. The MSE Prepspin 50 preparative ultracentrifuge operates at up to 50,000 r.p.m.

(347,000g) with a maximum capacity of 2,000 ml. The Prepspin features a new overspeed protection system, a wide range of angle and swing-out rotors in aluminium and titanium, full zonal capability, variable acceleration and braking, fast acceleration, automatic oil recirculation, and a brush-wear indicator. Also available are the Prepspin 65 and Prepspin 75 for operation at higher



MSE Prepspin 75

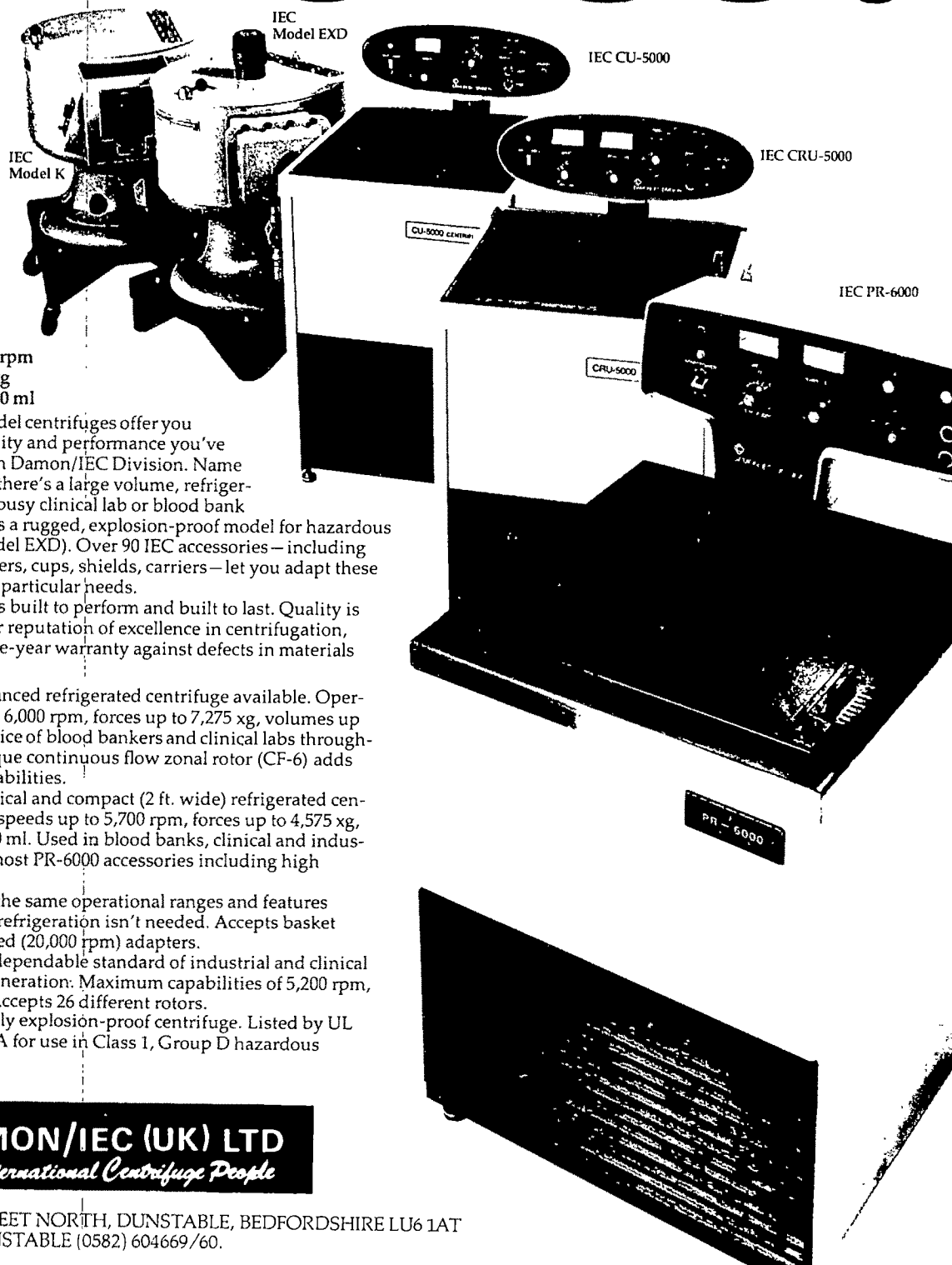
speeds. The Coolspin is a refrigerated centrifuge particularly suited for use in blood transfusion centres. The capacity of this centrifuge is 6 l at over 5,000g; it can accommodate 12 blood bags or carry 288 samples at one time. The Coolspin operates in the temperature range -20° to -40°C: acceleration and braking are variable and acceleration is fast. The range of rotors includes a 4×1,000 ml windshielded swing-out rotor developing 6,140g at 5,200 r.p.m., a two-place head for use in radio-immunoassay and autoanalyser work and the 'A' type zonal rotor. The Hi-Spin 21 is a high-speed 3-l capacity refrigerated centrifuge. The rotor complement includes large-capacity angle rotors such as the 6×500 ml and the 24×15 ml rotors, a continuous action rotor and the HS zonal rotor. To meet the demand for a simple and reliable refrigerated bench-top centrifuge MSE have produced the Chilspin. Chilspin has a maximum speed of 6,100 r.p.m. (6,030g) and uses the same rotors and accessories as the MSE Super Minor.

Circle No. 55 on Reader Enquiry Card.

Hettich. The Hettich EBA 3S is a newly designed bench-top laboratory centrifuge. The spin time can be set to 1-60 min, and the speed of rotation can be pre-selected and monitored on a revolution counter. Angular steel rotors are provided for either four or six buckets. Balancing is not essential because of the elastic suspension of the drive in the housing. The maximum rotor speed is 5,500 r.p.m.

Circle No. 56 on Reader Enquiry Card.

Choice.



Speeds up to 6,000 rpm
Forces up to 7,275 xg
Volumes up to 6,000 ml

These five floor model centrifuges offer you the versatility, quality and performance you've come to expect from Damon/IEC Division. Name your application—there's a large volume, refrigerated model for the busy clinical lab or blood bank (PR-6000), as well as a rugged, explosion-proof model for hazardous environments (Model EXD). Over 90 IEC accessories—including rotors, rings, adapters, cups, shields, carriers—let you adapt these centrifuges to your particular needs.

Each centrifuge is built to perform and built to last. Quality is backed by a 75-year reputation of excellence in centrifugation, along with a full one-year warranty against defects in materials or workmanship.

PR-6000. Most advanced refrigerated centrifuge available. Operates at speeds up to 6,000 rpm, forces up to 7,275 xg, volumes up to 6,000 ml. The choice of blood bankers and clinical labs throughout the world. Unique continuous flow zonal rotor (CF-6) adds ultracentrifuge capabilities.

CRU-5000. Economical and compact (2 ft. wide) refrigerated centrifuge. Capable of speeds up to 5,700 rpm, forces up to 4,575 xg, volumes up to 4,000 ml. Used in blood banks, clinical and industrial labs. Accepts most PR-6000 accessories including high speed adapters.

CU-5000. Provides the same operational ranges and features as CRU-5000 when refrigeration isn't needed. Accepts basket rotors and high speed (20,000 rpm) adapters.

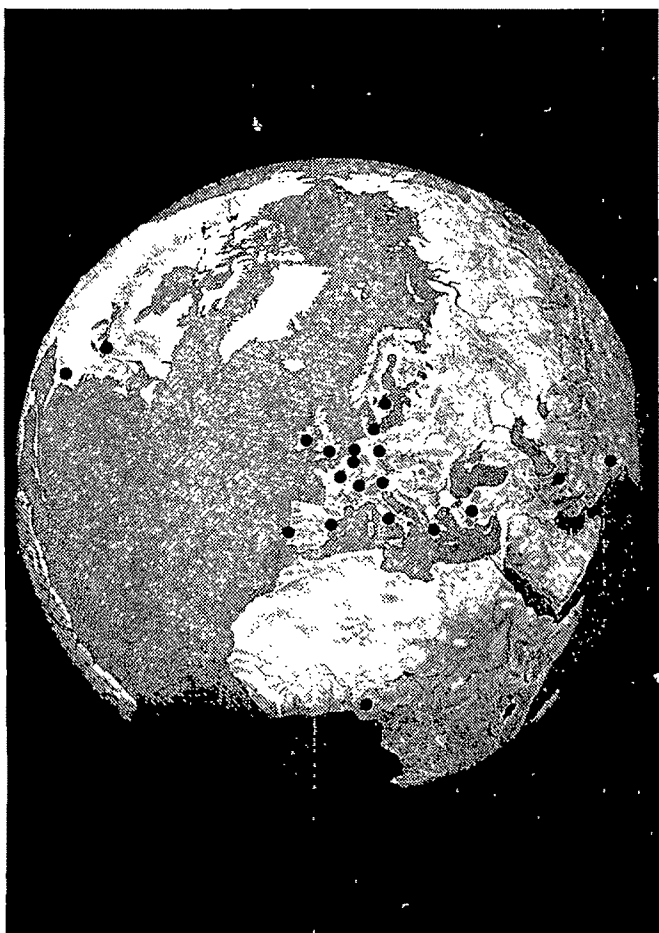
Model K. Rugged, dependable standard of industrial and clinical laboratories for a generation. Maximum capabilities of 5,200 rpm, 4,275 xg, 6,000 ml. Accepts 26 different rotors.

Model EXD. The only explosion-proof centrifuge. Listed by UL and certified by CSA for use in Class 1, Group D hazardous environments.

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MICROBIOLOGY Graduate seeks position involving work concerned with biological sciences or computing. Prepared to undertake further training as necessary. Box 1424 c/o Advertising Dept., Nature, 4, Little Essex Street, London WC2R 3LF. 1424(B)

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APPOINTMENTS VACANT

UNIVERSITY OF GLASGOW RESEARCH TECHNICIAN

A research technician with extensive laboratory experience and some training in immunological techniques is required to assist in a study of the immunoregulation of IgE production. The post, which is funded by the M.R.C., is available for 1 year in the first instance on Grade 3 of the University technicians salary scale (starting salary: £2,688).

Applications and enquiries to Dr E. Jarrett, Dept., Vet., Path., the University Veterinary Hospital, Bearsden, Glasgow G61 1QH (041-942 2301, Ext. 54).

In reply please quote Ref. No. 4083M. 1454(A)

UNIVERSITY OF TORONTO. Department of Mathematics. Tenure-stream appointments at the rank of **ASSISTANT PROFESSOR, in MATHEMATICS, APPLIED MATHEMATICS or HISTORY OF MATHEMATICS.** Appointments to commence July 1, 1978 subject to budgetary approval. Ph.D. and evidence of excellence in teaching and research required. Application should be made as soon as possible to: The Chairman, Department of Mathematics, University of Toronto, Toronto, Canada, M5S 1A1. 1445(A)

MEDICAL RESEARCH COUNCIL LABORATORY OF MOLECULAR BIOLOGY RESEARCH ASSISTANT

required to join a group working on chemical aspects of protein and oligonucleotide synthesis. Candidates should possess or expect to obtain a degree, H.N.C. or equivalent qualification in a science subject.

Salary on scale according to experience, starting from £2,823 plus 5% supplement. Good prospects for permanency and promotion to research officer grade.

Applications giving details of qualifications, experience, and the names of two referees, should be sent to:

Dr R. C. Sheppard, M.R.C. Laboratory of Molecular Biology, The Medical School, Hills Road, Cambridge CB2 2QH, as soon as possible. 1420(A)

KING'S COLLEGE HOSPITAL MEDICAL SCHOOL

(University of London)

Denmark Hill,

London SE5 8RX

LEUKAEMIA RESEARCH UNIT

(Department of Haematology)

RESEARCH TECHNICIAN

Graduate in Biological Sciences required to work in Cytogenetics Laboratory of a unit providing both diagnostic and research facilities for the study of chromosome abnormalities in Leukaemia.

The successful candidate will be responsible for running the cytogenetics laboratory and will also have the opportunity to participate in the various ongoing research projects of the department.

Some experience in cytogenetics preferred but full training will be given. Salary on Whitley Councils' Scale.

For further details, contact Dr J. C. Sharp or Mr A. W. Wayne 01-274 6222 extension 2013. Written applications, giving full curriculum vitae and the names and addresses of two referees to the Secretary of the Medical School at the above address.

Closing date March 3, 1978.

1466(A)

UNIVERSITY OF SASKATCHEWAN COLLEGE OF DENTISTRY SASKATOON, CANADA ORAL BIOLOGY

Applications are invited for two full-time positions in the Department of Oral Biology. Depending on qualifications, applicants may be considered for Chairmanship of the Department.

ORAL PATHOLOGY: The applicant should have a dental degree and graduate training in Pathology—Oral and/or General. Responsibilities will include teaching at the undergraduate level, research and managing a diagnostic biopsy service.

ORAL BIOCHEMISTRY/ MICROBIOLOGY: The applicant should have a dental degree and graduate training preferably beyond the Masters level. Responsibilities will include teaching at the undergraduate level and research in biochemistry/microbiology.

Rank and salary will be determined according to qualifications and experience.

Letters of application and curriculum vitae should be sent to:

Dr E. R. Ambrose, Dean,
College of Dentistry,
University of Saskatchewan,
Saskatoon, Saskatchewan,
Canada S7N 0W0 1442(A)

UNIVERSITY OF TORONTO, Department of Mathematics. Possible tenure-stream and/or contractually limited positions at the rank of **ASSISTANT PROFESSOR in STATISTICS,** on the St George and/or Erindale College campuses. Appointments to commence July 1, 1978. Ph.D. and evidence of excellence in teaching and research required. Application should be made as soon as possible to: The Chairman, Department of Mathematics, University of Toronto, Toronto, Canada, M5S 1A1. 1447(A)

HERIOT-WATT UNIVERSITY EDINBURGH

Department of Physics

Applications are invited for the following positions, which will be associated with an established group in the area of **SOLAR ENERGY CONVERSION** by Photovoltaic Cells based on amorphous semi-conductors fabricated by the glow discharge process. The work of the group is funded by S.R.C. and the E.E.C.

POST-DOCTORAL RESEARCH ASSOCIATE

Applicants should have relevant research experience in experimental Solid State Physics, materials science, or electronic device technology. The position will be tenable for two years in the first instance. Salary on Research Range 1A (£3,333 to £5,627, under review).

RESEARCH TECHNICIAN

Applicants should preferably have experience as a research technician and mechanical and/or electronic workshop experience. Duties will include assistance with preparation and testing of solar cells. Grade of appointment is flexible. The probable salary range is £2,688 to £3,720.

RESEARCH STUDENTSHIP

A Research Studentship is also offered in the above area. Informal enquiries may be made to:

Dr J. I. B. Wilson,
Department of Physics,
Heriot-Watt University,
Telephone: 031-449 5111, Ext. 2388

Application forms are available from the Staff Officer, Heriot-Watt University, Chambers Street, Edinburgh EH1 1HX. 1393(A)

FLOUR MILLING AND BAKING RESEARCH ASSOCIATION CHEMISTS AND BIOCHEMISTS STATISTICIAN/MATHEMATICIAN

The Flour Milling and Baking Research Association carries out a wide range of research and service work for the Milling and Baking Industries and holds a number of research contracts for the Ministry of Agriculture, Fisheries and Food. The laboratories are situated in an attractive semi-rural area on the edge of the Chilterns with ready access to London.

Several positions are available for graduates with strong research backgrounds who would be interested in carrying out basic and applied research on cereal problems.

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fundamental studies on cereal proteins

ORGANIC CHEMIST/BIOCHEMIST (two posts)

natural product chemistry

CHEMIST/ANALYTICAL CHEMIST

application of modern chemical/physical analytical techniques

BIOCHEMIST

nutritional and toxicological studies

STATISTICIAN/MATHEMATICIAN

statistical and mathematical service for the

Research Association

Applicants should have a Ph.D. degree and further research experience. For the post of Statistician/Mathematician a B.Sc. or equivalent or postgraduate qualification in statistics or mathematics together with several years applied experience is required.

Starting salary will depend on qualifications and experience within the Senior Scientific Officer (c. £4,350 to £5,810 p.a.) or Principal Scientific Officer (c. £5,620 to £7,120 p.a.) scales. There is a pension scheme and assistance with relocation expenses will be given where appropriate.

Further details can be obtained from Professor Brian Spencer, Director-General, Flour Milling and Baking Research Association, Chorleywood, Herts. WD3 5SH. Telephone: 092 78-4111. Applications, with the names and addresses of three referees, should be submitted by March 20. 1404(A)

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This is an opportunity for a young scientist — probably mid-late twenties — to make his or her mark within an R & D team which has an impressive record of innovation and successful implementation in a competitive sector of the consumer food market.

The job will appeal to commercially minded scientists capable of relating their specialist skills to the broader business implications of food research. The objective of the R & D team is to maintain a fundamental research programme into new raw materials, products and food technologies. There is considerable scope for you to develop your original ideas within the team — provided you have the communicative powers to present and "sell" them to technical and commercial

management.

You will have a good honours degree or PhD in Food Technology or Science, Biochemistry or Biochemical Engineering backed by formal training in research and preferably practical experience in the food industry.

Starting salary will be around £8,000 plus full large company benefits including assistance with relocation to an attractive part of the rural East Midlands.

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1455(A)

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MEDICAL RESEARCH COUNCIL LABORATORY ANIMALS CENTRE Carshalton, Surrey

Applications are invited for the post of Senior Research Officer in the Director's Department.

The successful candidate will be expected to assist the Director with:

- (i) personal assistance with his administrative duties
- (ii) a research programme designed to evaluate specific pathogen free cats.

It is hoped that he/she will be a mature person with an interest in scientific administration. Applicants should have a degree in biological science, together with a Ph.D., and previous experience with small animals would be an advantage although not essential. The post offers excellent conditions of service. Salary in the range £3,999 to £5,199 according to age, qualifications and experience, plus £354 London Weighting, plus £312 Pay Supplement, plus Variable Pay Supplement. Contributory Superannuation Scheme.

Applications, together with a curriculum vitae and the names of two referees should be sent to the Director, Medical Research Council Laboratory Animals Centre, Woodmansterne Road, Carshalton, Surrey SM5 4EF.

1377(A)

MRC

Medical Research Council

UNIVERSITY OF SURREY SCIENTISTS FOR MANAGEMENT/PRODUCT DEVELOPMENT

Applications are invited for the position of Research Officer or Research Fellow to work on a Department of Industry sponsored project for two years duration concerned with the development of novel printing inks designed to facilitate the recycling of waste papers.

Candidates (male or female) should possess a high but versatile level of practical skills together with a multi-disciplinary approach to problem solving, preferably with a background in chemistry, polymer science or paper technology. Applicants should, in addition, have management potential and be capable of developing contracts with government agency and private industry to further the interests of the project.

Salary according to age, experience, etc. will be on either the Research Range 1B: £2,904 to £4,190 p.a. or Range 1A: £3,333 to £5,423 p.a. (under review).

Applications should be sent as soon as possible to:-

Dr M. L. Burstall,
Department of Chemistry,
University of Surrey,
Guildford,
Surrey, GU2 5XH.

1437(A)

UNIVERSITY OF NAIROBI KENYA

Applications are invited for the following posts: DEPARTMENT OF VETERINARY CLINICAL STUDIES —PROFESSOR. Applicants should be in possession of a recognised Veterinary degree with a higher degree or its equivalent in one of the following areas: Veterinary Medicine, Surgery, Reproduction and Obstetrics or Veterinary Preventive Medicine. Candidates must also have considerable teaching and research experience at the University level. A knowledge of Tropical Veterinary Medicine will be an added advantage.

DEPARTMENT OF VETERINARY PATHOLOGY AND MICROBIOLOGY—SENIOR LECTURER. Applicants must be veterinarians and have a Ph.D. in Pathology or Microbiology and must have teaching experience in undergraduate and graduate levels in Veterinary Medicine especially in infectious diseases of domestic and wild animals. Salary scales: Professor K£4,632 to K£5,562 p.a. Senior Lecturer K£2,988 to K£3,984 p.a. (K£1=£1.30). The British Government may supplement salaries in range £4,128 to £4,674 p.a. (sterling) for married appointees and £2,880 to £3,384 p.a. (sterling) for single appointees (reviewed, annually and normally free of all tax) and provide children's education allowances and holiday visit passages. Terms of service include superannuation scheme, medical aid scheme, family passages and various allowances. Detailed applications (two copies) with curriculum vitae and naming three referees to be sent to Registrar, University of Nairobi, PO Box 30197, Nairobi, Kenya by March 7, 1978. Applicants resident in UK should send one copy to Inter-University Council, 90/91 Tottenham Court Road, London. W1P 0DT. Further details may be obtained from either address. 1433(A)

UNIVERSITY OF LONDON KING'S COLLEGE DEPARTMENT OF BIOCHEMISTRY

A full-time research position, financed by the Medical Research Council, is available for three years. The work will be concerned with the metabolism of messenger RNA during erythroid cell development.

Applicants should hold a Ph.D. degree, preferably in Biochemistry, and have previous experience of techniques relevant to nucleic acid isolation and cell-free protein biosynthesis.

Starting salary up to £3,569 per annum plus £450 per annum London Allowance plus provision for superannuation.

Applications, with full curriculum vitae and the names of two referees, should be sent as soon as possible to Professor H. R. V. Arnstein, Department of Biochemistry, King's College, The Strand, LONDON WC2R 2LS, from whom further information may be obtained, quoting reference 191603/N. 1465(A)

UNIVERSITY OF EDINBURGH DEPARTMENT OF MOLECULAR BIOLOGY

Applications are invited for an M.R.C. postdoctoral fellowship to work on the molecular relationships of plasmids, principally those carried by enterobacteria and *Pseudomonas* species from soil and clinical sources. A variety of biochemical and genetical techniques will be involved. These will include DNA-DNA hybridisation and probably heteroduplex analysis and the generation of recombinant plasmids both *in vivo* and *in vitro*. The appointment is for three years from April 1978 or when convenient thereafter. The starting salary will be between £3,333 and £4,190 p.a. as appropriate.

Applications with curriculum vitae and the names and addresses of two referees should be sent to Dr P. M. A. Broda, Department of Molecular Biology, King's Buildings, Mayfield Road, Edinburgh, EH9 3JR. Please quote Reference 5001. 1436(A)

THE UNIVERSITY OF LEEDS

DEPARTMENT OF PATHOLOGY

Applications are invited for the following posts available from October 1, 1978:

LECTURER/ SENIOR LECTURER (ref. 102/2/D)

Applications are invited from registered medical practitioners. Duties will include teaching, research and health service work. A special interest in one of the main branches of histopathology, such as cardiac, respiratory, skeletal, dermatological, or oncology is desirable but not essential. The successful applicant will be appointed at the level most appropriate to experience and qualifications, and the AHA(T) will be asked to award honorary consultant status or other appropriate grade to a suitably qualified individual.

Salary on the scale for Honorary Consultants £7,536 (plus supplements of £312 and £208) to £8,327 (plus supplements of £178 and £208) to £9,111 (plus supplement of £208) to £9,900 (plus supplement of £208) to £10,689 (plus supplement of £208), or on the scale for Senior Clinical Lecturers £6,915 to £8,142 (plus supplement of £312) (under review), or on the scale for Clinical Lecturers £3,742 to £7,440 (plus supplement of £312) (under review).

The Department may be visited by arrangement with Professor C. C. Bird.

LECTURER (ref. 102/3/D)

Applications are invited from medical practitioners. Previous postgraduate experience in histopathology is desirable but not essential. Duties will include teaching, research and health service work. The AHA(T) will be asked to award a suitable honorary clinical contract.

Salary on the scale for Clinical Lecturers £3,742 to £7,440 (plus £312 supplement) (under review).

Application forms and further particulars for both posts may be obtained from the Registrar, The University, Leeds LS2 9JT, quoting appropriate reference number. Closing date for applications March 10, 1978. 1399(A)

Young qualified **TECHNICIAN** required in new dermatology research laboratory in Clinical Science Laboratories, Guy's Tower, for investigative work in histopathology, histochemistry and electron microscopy. Experience in preparative techniques in electron microscopy and use of the transmission electron microscope essential; training in pathology an advantage. Appointment for one year in first instance from April 1, 1978. Initial salary in range £2,982 to £3,379 according to qualifications and experience, plus £354 London Weighting and superannuation. Applications with names of two referees to the Secretary, Guy's Hospital Medical School, London Bridge, SE1 9RT, quoting Ref. D.R.1. 1435(A)3

KINGSTON POLYTECHNIC

Appointment of PRINCIPAL LECTURER in GEOLOGY

The School operates a range of C.N.A.A. degree courses and is actively engaged in research. A good background of research is desirable, whilst industrial and/or survey experience would be an advantage.

Salary range including 1976 and 1977 supplements £6,432 to £7,134 (bar) to £8,070 plus £297 London allowance.

Further details and application forms (to be returned by March 6) from Academic Registry, Dept. AO, Kingston Polytechnic, Penrhyn Road, Kingston upon Thames KT1 2EE. 01-549 1366. 1449(A)

IMPORTANT INTERNATIONAL PHARMACEUTICAL GROUP

located in PARIS is presently offering an **EXCELLENT CAREER OPPORTUNITY** for a

BIOCHEMIST/PHARMACOLOGIST

to join in the **DEPARTMENT OF BIOLOGICAL RESEARCH THE METABOLIC AND ENDOCRINE DISEASE UNIT** as

PROJECT LEADER

for:

- the **DISCOVERY** and **EVALUATION** OF NEW DRUGS
- the **DRAFTING** AND **IMPLEMENTATION** OF NEW PRODUCTS IN **BASIC RESEARCH**.

Preference will be given to individuals with the following qualifications:

- Ph.D. degree
- postdoctoral or industrial research experience (3-5 years)
- good attitude towards team work
- age between 30 and 40 years.

An excellent salary and comprehensive benefit programme accompany this position.

Please send curriculum vitae to Box 1272, Nature Magazine, c/o Macmillan Journals Ltd., 4 Little Essex Street, London WC2R 3LF. 1272(A)

KING'S COLLEGE HOSPITAL MEDICAL SCHOOL (University of London)

Denmark Hill, London SE5 8RX

DEPARTMENT OF CHILD HEALTH AND LIVER UNIT RESEARCH ASSISTANT IN CELL-MEDIATED IMMUNITY

Applications are invited for the above post to participate in a study of liver disease in subjects with cystic fibrosis. An Honours Degree in biology or biochemistry and previous experience in immunology desirable.

Salary from £3,377 per annum.

Applications, with a curriculum vitae and the names of two referees should be sent to the Secretary, King's College Hospital Medical School, from whom further details are available. Closing date for applications March 3, 1978. 1431(A)

INSTITUTE OF CANCER RESEARCH ROYAL CANCER HOSPITAL

A Constituent of the British Postgraduate Medical Federation, University of London

Director of the Institute

The Committee of Management has appointed a Sub-Committee to recommend a successor to the present Director of the Institute. The new Director will take up the appointment on a date to be negotiated.

The Sub-Committee invites applications for the post. Any person with experience of cancer research may be considered, irrespective of field of work. A medical qualification is desirable but not essential. Enquiries and applications should be made to the Chairman (Professor Sir Richard Doll, OBE., FRS.) at the office of the Secretary.

The Committee of Management reserves the right to appoint to the post by invitation.

Information about the Institute and further particulars of the post may be obtained from the Secretary, 34 Sumner Place, London, SW7 3NU. Applications should be submitted before 14 April, 1978 (1422)A



Open to both
men and women

Public Service Commission
Canada
Fonction publique
Canada

RESEARCH SCIENTISTS — MARINE MAMMALOGISTS (2 positions)

Initial salary up to \$36,760 (dependent on experience)
Ref. No: 77-PSTP-32-155 (N)

Fisheries and Environment Canada
St. John's, Newfoundland

Duties

Two research scientists are required in the Marine Fisheries Management Program to conduct independent research into the biology, ecology and population dynamics of marine mammals in the North-West Atlantic area. One position involves work on the harp and hood seals and the other involves the cetacean species. The selected scientists will organize and participate in field surveys for the collection of data and specimens for investigations; and will analyse data, interpret variables and present results to the scientific community and resource managers in order that the latter may formulate policies for the rational use of each species.

Qualifications

Applicants must possess a Ph.D. or a lesser degree with evidence of research experience and productivity equivalent to that of the Ph.D. in the field of biology. Experience in the fields of marine mammalogy and population biology is also required. Knowledge of English is essential.

How to Apply

Send application form and/or résumé to:

Professional, Scientific and Technical Program
Public Service Commission of Canada

Ottawa, Ontario K1A 0M7

Closing Date: March 20, 1978

1406(A)

Please quote the applicable reference number at all times.

nature

Nature is considering employing a physical scientist to assist Dr Stuart Sharrock, the Physical Sciences Editor. Such a person would have recently received a Ph.D. and would be hoping to develop a much broader appreciation of science. He or she would be expected to help Dr Sharrock in the assessment of manuscripts, work with other members of the *Nature* staff in compiling the various sections of the journal, establish contacts in the scientific community and, on occasions, write short pieces on recent scientific developments.

Write to Editor, *Nature*, 4 Little Essex Street, London WC2 enclosing a c.v. and any other material you consider relevant.

1430(A)

UNIVERSITY OF THE WEST INDIES JAMAICA

Applications are invited for the following posts: PROFESSOR/SENIOR LECTURER IN APPLIED MATHEMATICS. The appointee will be a person specialising in Modern Applied Mathematics with teaching and research experience in Operational Research or Statistics or Control Theory and allied fields.

SENIOR LECTURER/LECTURER IN APPLIED MATHEMATICS. The appointee will be a person with teaching and research experience in Statistics or allied fields.

LECTURER / ASSISTANT LECTURER IN PHYSICAL CHEMISTRY. Applicants should have interests in either Photochemistry, Electrochemistry, Radiochemistry or Gas Phase Kinetics. Salary scales: Professor: 1977/78—J\$17,166 to J\$21,252 p.a. Senior Lecturer: 1977/78—J\$12,066 to J\$15,897 p.a. Lecturer: 1977/78—J\$8,913 to J\$ 13,917 p.a. Assistant Lecturer: 1977/78—J\$7,236 to J\$8,412 p.a. (£1 sterling=J\$2.42). F.S.S.U. Study and Travel Grant. Unfurnished accommodation will be let by the University at a rental of 10 per cent of salary, or a housing allowance of 20 per cent of salary will be paid. Family passages. Detailed applications (two copies) with curriculum vitae and naming three referees should be sent as soon as possible to the Registrar, UWI, Mona, Kingston, Jamaica. Further particulars are available from the same source or from the Inter-University Council for Higher Education Overseas, 90/91 Tottenham Court Road, London W1P 0DT. 1434(A)

UNIVERSITY COLLEGE DUBLIN

DEPARTMENT OF BIOCHEMISTRY

Applications are invited for an academic post at the level of either Assistant Lecturer or College Lecturer in Biochemistry. Applications would be particularly welcomed from candidates with experience in any of the following fields—proteins, nucleic acids, immunochemistry. The appointment will be tenable from October 1, 1978.

The current salary scales are:

Assistant Lecturer: £3,661 to £6,081

College Lecturer: £5,840 to £7,544

Entry point on the relevant scale will be in accordance with qualifications and experience. Family allowances or equivalent will be paid in addition to the above scale.

There is a non-contributory pension scheme. An alternative contributory F.S.S.U. type scheme is also available. Prior to application, further information (including details of application procedure) should be obtained from Mr J. P. MacHale, Secretary and Bursar, University College, Belfield, Dublin 4. Telephone: 693244, Ext. 431.

The latest date for receipt of completed applications is Thursday, March 16, 1978. 1409(A)

UNIVERSITY OF SURREY DEPARTMENT OF BIOCHEMISTRY EXPERIMENTAL OFFICER IN NUTRITION

There is a vacancy for an Experimental Officer to work with the Professor of Human Nutrition, Professor J. W. T. Dickerson. Candidates should have a good honours degree in a relevant subject and preferably some postgraduate experience. A strong interest in nutritional research is essential.

Salary will be within the Experimental Officer range: £3,975 to £5,015 per annum, depending on age and

Further particulars and application forms may be obtained from the Assistant Secretary (Personnel), University of Surrey, Guildford, Surrey GU2 5XH, or telephone Guildford 71281. Ext. 452. Completed forms should be returned by March 2, 1978. 1401(A)

THE SCHOOL OF PHARMACY UNIVERSITY OF LONDON LABORATORY SUPERINTENDENT

LABORATORY SUPERINTENDENT (Grade 8) required as soon as possible to be responsible to the Professor and Head of Department of Pharmaceutics for the organisation of laboratory services for teaching and research and the supervision of 22 technical and ancillary staff. The successful applicant should have experience in financial planning and stock control.

The Department comprises Physical Pharmacy, Microbiology and Pharmaceutical Engineering Science. Applicants should possess H.N.C. or equivalent qualification, and have 10-15 years experience in similar or related laboratories. Experience in workshop and personnel administration would be advantageous.

Salary Scale £4,594 to £4,987 plus London Weighting £465 per annum.

Applications in writing, giving full details of experience, qualifications and names of two referees to:

The Personnel Officer, The School of Pharmacy, 29/39 Bunswick Square, London WC1N 1AX. 1469(A)

UNIVERSITY OF CAMBRIDGE DEPARTMENT OF PHARMACOLOGY

Applications are invited for an office of:

UNIVERSITY LECTURER IN THE DEPARTMENT OF PHARMACOLOGY

The initial appointment will be for three years from October 1, 1978, with the possibility of re-appointment to the retiring age.

The pensionable stipend for a University Lecturer is on scale £4,607 to £7,087 a year, with initial placing above the minimum where appropriate. There is no grade of Senior Lecturer. A grant is made towards removal expenses.

Candidates should send twelve copies of their application together with the names of not more than three referees to Mr G. R. Anderson, General Board Office, The Old Schools, Cambridge CB2 1TT, from whom further particulars can be obtained, to arrive not later than March 20, 1978. 1417(A)

AVON AREA HEALTH AUTHORITY (T)— SOUTHMEAD HEALTH DISTRICT

DEPARTMENT OF CLINICAL CHEMISTRY

SOUTHMEAD HOSPITAL, BRISTOL

Applications are invited for a training post as a Basic Grade Biochemist, in connection with the University of Surrey M.Sc. Course in Clinical Biochemistry. Suitable candidates would hold, or would be expecting to obtain, a 1st or 2nd Class Honours degree in an appropriate science.

The appointment is for 3 years, the first 2 of which are spent on the M.Sc. Course, attending at Guildford for alternate University terms. The salary would be within the Whitley Council scales and the trainee is responsible for paying the University Course fees.

Further details may be obtained from Dr D. J. Goldie or Dr J. B. Holton at the above address (or Bristol (0272) 622821) and application forms are available from the District Personnel Officer, Southmead Hospital, Westbury-on-Trym, Bristol BS10 5NB (Tel: Bristol (0272) 622821 Ext. 162).

Closing date for applications: March 4, 1978. 1413(A)

UNIVERSITY OF WARWICK
POSTDOCTORAL
RESEARCH FELLOW
in VIROLOGY

Applications are invited for a Post-doctoral Fellowship with the Virus Research Group in the Department of Biological Sciences. This post is concerned with the use of isolated nuclei to study molecular events occurring during influenza virus multiplication. Experience in virology, cell biology and techniques for analysing proteins and RNA would be an advantage. Initial salary will be on the Research Range 1A scale £3,333 to £5,627 per annum (under review). The Fellowship is funded by the Cancer Research Campaign and is available immediately; it is then renewable on an annual basis until September 1980. Further details and application forms from the Academic Registrar, University of Warwick, Coventry CV4 7AL, quoting Ref. No. 27/R/78. Closing date for receipt of applications March 10, 1978. 1408(A)

UNIVERSITY OF
EAST ANGLIA
SENIOR
RESEARCH ASSOCIATE

required for two years to work with Dr G. S. Boulton, School of Environmental Sciences and Dr L. W. Morland, School of Mathematics and Physics in an investigation of the interactions between glaciers and underlying deformable materials. Applicants should have a good grounding in theoretical mechanics or soil mechanics theory and preferably some experience in numerical methods. There would also be the possibility of field work for an interested person. Applicants should have completed a Ph.D. in mathematics or other related disciplines. The appointment would be made at the appropriate age-point within the salary range £3,333 to £5,219 p.a. (under review). Applicants should send a curriculum vitae and the names of two referees to Dr G. S. Boulton, School of Environmental Sciences, University Plain, Norwich NR4 7TJ. No forms of application are issued. 1450(A)

UNIVERSITY OF
GLASGOW
LECTURESHIP IN
GEOLOGY

Applications are invited for a lectureship in structural geology. Preference will be given to those combining field and theoretical studies. Interest in teaching Engineering geology or ore geology would be an advantage. The initial salary will be within the range £3,333 to £4,811 per annum on the Lecturers' scale of £3,333 to £6,655 per annum according to qualifications and experience. Appropriate Superannuation Scheme will apply.

Further particulars may be had from the Secretary of the University Court (Room 18). The University of Glasgow, Glasgow G12 8QQ, with whom applications (8 copies) giving the names and addresses of three referees, should be lodged on or before March 20, 1978.

In reply please quote Ref. No. 4081M. 1414(A)

GRADUATE ASSISTANT

A position is available to work in a group on metabolic aspects of connective tissue diseases. The candidate must have a good background in biochemistry, but experience is not essential.

The starting salary is up to £3,333 per annum, and carries U.S.S. benefits. The position is available for three years. Please send curriculum vitae and names of two referees to The Secretary, Srageways Research Laboratory, Wort's Causeway, Cambridge; 1403(A)

OVERSEAS DEVELOPMENT

KNOW-HOW: vital to developing countries

Agricultural Statistician

Lesotho

To collect, process, analyse and publish agricultural statistics and design agricultural surveys. Applicants should be qualified Statisticians with background in sampling and experience in collection and analysis of Agricultural Statistics.

Appointment 2 years. Salary (UK taxable) to be arranged plus tax-free overseas allowance in range £1025—£2190 pa. (Ref 328X).

Extension Agronomist

Sudan

At Nuba Mountains Agricultural Production Corporation to assist with implementation of field programmes. Applicants should have University degree in Agriculture or related science, with at least 5 years' experience of crop production and extension work in developing country.

Appointment 3 years. Salary in range £7000—£8500 pa plus tax-free overseas allowance in range £1755—£4555 pa. (Ref 331X).

The posts are wholly financed by the British Government under Britain's programme of aid to the developing countries. In addition to basic salary and overseas allowance other benefits normally include paid leave, free family passages, childrens education allowances and holiday visits, free accommodation and medical attention. Applicants should be citizens of the United Kingdom.

For full details and application form please apply, quoting reference stating post concerned, and giving details of age, qualifications and experience to:—



Appointments Officer,
MINISTRY OF OVERSEAS DEVELOPMENT,
Room 301, Eland House,
Stag Place, London SW1E 5DH.

1412(A)

HELPING NATIONS HELP THEMSELVES

UNIVERSITY OF THE
WEST INDIES—TRINIDAD

Applications are invited for the post of LECTURER/ASSISTANT LECTURER in CROP PRODUCTION, FACULTY OF AGRICULTURE. Applicants should possess a higher degree in Agriculture or Plant Sciences and experience in teaching and research in the tropical field or horticultural crops. Preference will be given to candidates with knowledge of and experience in Crop Production systems practised in the Caribbean Region. The applicant will be expected to assume duties not later than July 1978.

Salary Scales: 1977/78: Lecturer: TT\$19,071 to TT\$29,799 p.a. Assistant Lecturer: TT\$15,480 to TT\$16,974 p.a. (£1 sterling = TT\$4.66) F.S.S.U. Unfurnished accommodation if available at 10% or furnished at 12½% or housing allowance of 10% of pensionable salary. Up to five full economy passages on appointment and on normal termination. Study and Travel Grant. Detailed application naming three (3) referees to Secretary, U.W.I. St Augustine, Trinidad. Details of post sent to all applicants. 1460(A)

UNIVERSITY OF TORONTO. Department of Mathematics. PROFESSOR. Demonstrate record of scholarly achievement. Teaching graduate and undergraduate courses. Direction of Ph.D. theses and otherwise maintaining a high standard of research in the Department. Salary negotiable, subject to budgetary approval. Effective date of appointment July 1, 1978. Application should be made as soon as possible to: The Chairman, Department of Mathematics, University of Toronto, Toronto, Canada, M5S 1A1. 1446(A)

UNIVERSITY OF
TASMANIA

LECTURESHIP/SENIOR
LECTURESHIP IN
PATHOLOGY

Applications are invited for this position with the salary scale as follows:

Senior Lecturer—\$A19,971 to 4 by 666 to 1 by 664 to \$23,299; Lecturer—\$A14,851 to 3 by 672 to 4 by 671 to \$19,551. An appointment will be offered from the beginning of 1979 within one of these grades, according to qualifications and experience. An additional loading is paid to medical staff with clinical responsibility. For this appointment, it is expected that the loading would be \$A5,000 p.a.

Applicants must have had experience in the practice of pathology and the successful appointee will be expected to contribute to the Pathology services of the Royal Hobart Hospital, where he will be given an appropriate appointment.

Intending applicants are invited to write to Professor R. A. Rodda, Department of Pathology in the University, for any further information they may require.

The University reserves the right to offer a short-term contract appointment, or a probationary appointment, rather than an appointment with immediate tenure. Conditions of appointment and application forms obtainable from Association of Commonwealth Universities (Appts). 36 Gordon Square, London WC1H 0PF. Applications close on March 31, 1978. 1427(A)

UNIVERSITY OF NAIROBI
KENYA

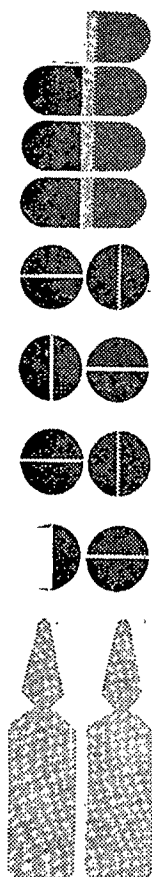
Applications are invited
for the post of

SENIOR LECTURER

in the

DEPARTMENT OF ZOOLOGY

Applicants should be zoologists with considerable teaching and research experience in ecology, vertebrate zoology, palaeontology, cell biology or immunology. Outstanding candidates with qualifications in other fields of Zoology will also be considered. The Department's main teaching and research interests are in ecology, comparative physiology, parasitology and hydrobiology (marine, freshwater and fish biology). Salary scale: K£2,988 to K£3,984 per annum (K£1=£1.30). The British Government may supplement salary in range of £4,128 per annum (sterling) for married appointee or £2,880 per annum (sterling) for single appointee (reviewed annually and normally free of all tax) and provide children's education allowances and holiday visit passages. Terms of service include superannuation scheme; medical aid scheme; family passages and various allowances. Detailed applications (two copies) with curriculum vitae and naming three referees to be sent to Registrar, University of Nairobi, PO Box 30197, Nairobi, Kenya by March 7, 1978. Applicants resident in UK should send one copy to Inner University Council, 90/91 Tottenham Court Road, London W1P 0DT. Further details may be obtained from either address. 1407(A)



New Product Planning

Graduate Scientist

An opportunity has arisen for a young scientist to assist in the planning and co-ordination of the development of new medicinal products from the time of their identification to marketing.

The job involves preparing plans for the development of compounds, compiling and maintaining data on development projects and liaising between departments within the Company. He or she will act as secretary to key committees and be responsible for the production and distribution of appropriate documents for these committees.

Candidates will be graduates in a scientific discipline relevant to the pharmaceutical industry. Ideally, they will have some experience in a research environment and the ability to co-operate and communicate effectively with all levels and disciplines. 1978 Graduates will probably have insufficient experience for the position.

The post carries a competitive salary which will reflect the age and experience of the successful applicant; other benefits include pension and profit sharing schemes, and assistance with relocation expenses where appropriate to this very pleasant part of Hertfordshire.

Applications to: Dr I. Collins, Personnel Manager, Allen & Hanburys Research Ltd., Ware, Herts SG12 0DJ. Tel: Ware 3232. 1470(A)



**Allen & Hanburys
Research Limited**



SCIENCE RESEARCH COUNCIL ROYAL GREENWICH OBSERVATORY ASTROPHYSICS DIVISION

The Observatory has a vacancy for a Higher Scientific Officer to initiate and carry out observing programmes and theoretical interpretation of data in one or more of the following fields:

Stellar evolution, chemical composition of stars and stellar populations, galactic and extra galactic HII regions, star clusters, the structure and dynamics of normal and peculiar galaxies and of clusters of galaxies.

Observations will be made using Science Research Council and other facilities in the U.K. and overseas.

In addition the person appointed is likely to be involved in the design of instruments for astronomical research and in assisting visiting observers with observing and data reduction procedures.

Qualifications: Candidates should have a Ph.D. degree or equivalent qualification preferably with some post-doctoral experience in astrophysical research at a major observatory.

Salary: According to experience, within a range £3,745 to £4,976 per annum including supplements. The Council has a non-contributory pension scheme.

Application forms are available from: Mr J. Philcox, Royal Greenwich Observatory, Herstmonceux Castle, Hailsham, East Sussex BN27 1RP, telephone Herstmonceux 3171 and should be returned not later than March 24, 1978. 1472(A)

THE UNIVERSITY OF LEEDS DEPARTMENT OF MEDICAL PHYSICS

Applications are invited for the post of LECTURER in the above Department. Preference will be given to applicants already holding doctorate research qualifications and having experience in aspects of radiation physics.

Salary on the lecturer scale £3,333 to £6,655 (under review).

Application forms and further details may be obtained from the Registrar, The University, Leeds LS2 9JT, quoting reference number 92/1/D. Closing date for applications March 17, 1978. Informal enquiries may be addressed to Professor R. E. Ellis, Department of Medical Physics, The General Infirmary, Leeds LS1 3EX. Tel: Leeds 32799, ext. 487. 1415(A)

THE UNIVERSITY OF LEEDS DEPARTMENT OF PHYSIOLOGY

Applications are invited for a post of TEMPORARY LECTURER in the above Department. The post is tenable for one year, and is available now. The Department has Science, Medical and Dental teaching commitments, and research interests in the major fields of mammalian physiology. Medical qualification would be welcome.

Salary in the range £3,333 to £4,403 (under review) of the scale for Lecturers.

Further details may be obtained from the Registrar, The University, Leeds LS2 9JT, quoting reference number 104/5/D. Closing date for applications March 10, 1978. 1416(A)

THE UNIVERSITY OF LEEDS ASSISTANT LIBRARIAN

Applications are invited for a post of Assistant Librarian in the University Science Library. Candidates must be graduates in science or applied science with professional library qualifications or research experience.

Salary will be on the IA Scale for Senior Library Staff £3,333 to £5,627 (under review).

Application forms and further particulars may be obtained from the Registrar, The University, Leeds LS2 9JT, quoting reference number 116/7/D. Closing date for applications March 10, 1978. 1467(A)

UNIVERSITY OF EDINBURGH FACULTY OF MEDICINE LECTURER IN PHARMACOLOGY

Applications are invited for the post of Lecturer in the Department of Pharmacology as from October 1, 1978. This is a full-time appointment in which the successful candidate will be expected to make a research contribution. Salary on the scale £3,333 to £6,655. U.S.S. superannuation.

Applications (six copies) giving the names of two referees should be sent, not later than March 17, 1978, to the Secretary to the University, Old College, South Bridge, Edinburgh EH8 9YL, from whom further particulars may be obtained. Please quote Reference 1011. 1418(A)

BIOCHEMIST

An opportunity exists in this Pharmaceutical Research Company for a recent Honours Graduate in Biochemistry to join a small team involved in the preparation of enzymes from biological materials.

Some experience of protein purification techniques is desirable but a capacity to work with a minimum of supervision after training is of greater importance.

Application forms are available from The Company Secretary, Biorex Laboratories Ltd., Biorex House, Canonbury Villas, London N1 2HB. 1394(A)

ROYAL FREE HOSPITAL SCHOOL OF MEDICINE (University of London)

A TECHNICIAN is required to work in the Academic Department of Surgery at the Royal Free Hospital, Hampstead. Experience in tissue culture and biochemistry desirable. Qualifications—H.N.C. or equivalent. Salary on Whitley Council Scale.

Application forms available from The School Secretary, R.F.H.S.M., 8 Hunter Street, London WC1N 1BP (or telephone 01-837 5385. Ext. 8 or 10). Closing date: March 2, 1978. 1439(A)

ST GEORGE'S HOSPITAL MEDICAL SCHOOL (University of London) RESEARCH ASSISTANT (IMMUNOLOGY)

A research assistant is required in the new section of Immunology to work on aspects of human 'in vitro' cellular immunology. This post would be suitable for some with laboratory experience in immunology or a related subject and who is able to carry out research projects without close supervision. For further details apply with curriculum vitae to Sector Administrator, Department of Biochemistry, St George's Hospital Medical School, Cranmer Terrace, London SW17 0RE. 1396(A)

UNIVERSITY MARINE
BIOLOGY STATION
MILLPORT
RESEARCH ASSISTANT

is required to work on the behaviour of subtidal burrow building macrofauna under the direction of Dr R. J. A. Atkinson. The appointment (N.E.R.C. supported) is for two years from March 1, 1978 or as soon thereafter as possible. Salary £2,904 with one increment of £215 after the first year.

Applicants should have an honours degree in Zoology or Biology and diving qualifications to B.S.A.C. 2nd Class or equivalent.

Applications (three copies) together with the names of three referees, one of whom has personal knowledge of diving ability, should be received no later than February 24, 1978 by the Secretary, University Marine Biological Station, Millport, Isle of Cumbrae, Scotland KA28 0EG. 1395(A)

SWISS FEDERAL
INSTITUTE OF
TECHNOLOGY (ZURICH)
RESEARCH ASSOCIATE OR
FELLOW IN
BIOCHEMISTRY

Applications are invited for the post of Research Associate or Fellow to work on a project concerning protein sequence determinations. Experience in biochemistry, especially in the techniques of protein sequence determination is essential. A Ph.D. or equivalent is preferable. The project is supported for 2 years by a grant of the Swiss Federal Institute of Technology. The salary will be SFr. ~40,000.-/year (\$ ~20,000.-/year, £ ~15,000.-/year).

Applications, including the names of two referees, should be sent to Professor Dr K. H. Winterhalter, Labor für Biochemie I, Universitätstr. 16, 8092 Zurich, from whom further details may also be obtained. Please quote Reference 1308 (A).

UNIVERSITY OF
BIRMINGHAM
DEPARTMENT OF
BIOCHEMISTRY

Applications are invited for a post of:

LECTURER IN
BIOCHEMISTRY

tenable from October 1978, or earlier. The successful candidate will be expected to participate in the general and specialised teaching programmes of the Department which provides courses for science, medical and dental undergraduates. Excellent facilities are available for most types of biochemical research.

Salary within the range £3,333 to £4,403 p.a. (plus U.S.S.), depending on age, qualifications and experience.

Applications (3 copies) naming 3 referees should be sent by March 6, 1978 to the Assistant Registrar (Senate), University of Birmingham, P.O. Box 363, Birmingham B15 2TT, from whom further particulars may be obtained. 1311(A)

POSTDOCTORAL
RESEARCH POSITION

Two-year appointment available for recent Ph.D. in Physiology or Biology to work on oxygen transport to the eye and retina. Will also be involved in other aspects of retinal physiology. Background or knowledge of electrophysiology helpful.

Excellent salary and benefits.

Send resume to John J. Weiter, M.D., Department of Ophthalmology, Tufts New England Medical Center, 171 Harrison Avenue, Boston, Mass. 02111.

An Equal Opportunity Employer, M/F. 1411(A)



Wellcome

Production Manager/ Senior Scientist

... Reagents Bulk Production

This is a senior appointment with responsibility for the day-to-day running of the Bulk Production areas; producing a complete range of *in vitro* diagnostic reagents covering Immunology, Bacteriology, Serology and Clinical Chemistry.

Reporting to the Production Manager, Wellcome Reagents Limited, the successful candidate will require extensive scientific and managerial skills to maintain and improve on present production performance, both by the application of approved management techniques and the introduction of improved production methods.

Candidates, probably not less than 35 years old, will hold a first (or higher) degree or equivalent qualification, and be able to demonstrate previous experience in the preparation and use of *in vitro* biological diagnostic reagents.

Salary will be commensurate with ability and experience, and will attract those presently earning in excess of £5,500 p.a.

The Wellcome Research Laboratories are situated in pleasant parkland surroundings within easy reach of Bromley and Beckenham and about 12 miles from Charing Cross. Conditions of employment are attractive and include four weeks' holiday, pension and sick pay schemes, subsidised canteen and excellent sports and social facilities and generous assistance with relocation expenses where appropriate.

Please write giving brief details of qualifications and experience to R. V. Sutton, Personnel Officer, The Wellcome Research Laboratories, Langley Court, Beckenham, Kent BR3 3BS, quoting reference no. U.852.

1471(A)



HEAD OF DIVISION
OF IMMUNOGENETICS

A vacancy exists for the post of HEAD OF DIVISION OF IMMUNOGENETICS (Salary according to AH3). The successful applicant will be responsible for teaching immunogenetics and serogenetics. The applicant should have had experience in the field of transplantation antigens in order to be able to perform antigen typing within the framework of hereditary biological diagnosis. The Institute promotes research in the field of mammalian development. The experimental work of the applicant should therefore be mainly directed to problems of the cell surface during development.

Applications, together with selected reprints should be sent before April 1, 1978 to:

Dekanat der Medizinischen Fakultät der Universität Göttingen
Wilhelmsplatz 2
3400 Göttingen
West Germany 1392(A)

POSTDOCTORAL FELLOW
MICROBIOLOGY

Applications are invited for a post-doctoral fellow to work on structure and genetics for influenza virus. Preference will be given to applicants with experience in the field of RNAs. Please send curriculum vitae and references to: Dr Peter Palese, Microbiology Department, The Mount Sinai School of Medicine, 100 Street and Fifth Avenue, New York, N.Y. 10029, USA. An equal opportunity employer. 1405(A)

THE UNIVERSITY OF
CALIFORNIA
Faculty position in
AQUACULTURE
ASSISTANT OR
ASSOCIATE PROFESSOR

Twenty per cent. teaching; 80 per cent. research. Ph.D. in a specific area of fisheries biology, with strong background in larval rearing of fish with species endemic to the Sacramento River, California, and interest in developing research and teaching in fish aquaculture. Interest in species other than salmonids preferred. Teaching at both undergraduate and graduate levels expected. Applicants should submit résumé, publication list, letter describing interests, three letters of reference, and for recent Ph.D.s, undergraduate and graduate transcripts to: GRAHAM A. E. GALL, ASSOCIATE DIRECTOR AQUACULTURE, DEPARTMENT OF ANIMAL SCIENCE, UNIVERSITY OF CALIFORNIA, DAVIS, CA 95616 PRIOR TO APRIL 30, 1978. Position available July 1, 1978. The University of California is an Equal Opportunity/Affirmative Action Employer. 1456(A)

PART-TIME
RESEARCH NURSE

20 hours per week to assist in research into gastro-intestinal and vascular disorders. Previous research experience not necessary. For further details and informal visit call St James' Hospital, Batham. 672-1222 Ext. 132. 1461(A)

UNIVERSITY OF
QUEENSLAND
Australia
LECTURER IN
VETERINARY ANATOMY

Applicants should hold appropriate academic qualifications for the position, should have previous experience in teaching and research and should present evidence that their teaching and research ability is of the highest quality. Preference will be given to applicants who hold a qualification in veterinary science. The successful applicant will be expected to teach the gross anatomy of domesticated animals as well as embryology and histology. March 20, 1978.

Salary: \$A14,851 to \$A19,551 per annum. Other Benefits: Study Leave, Superannuation, housing assistance, travel and removal expenses.

Additional information and application forms are obtainable from the Association of Commonwealth Universities (Acpus), 36 Gordon Square, London WC1H 0PF. 1428(A)

JUNIOR TECHNICIAN

preferably with some experience of cell culture and virology, required in Department of Microbiology. Salary on scale £2,044 to £2,654, or £2,155 to £2,758, according to age and qualifications, plus £354 London Weighting.

Apply in writing, stating age, and giving details of qualifications and experience, to the Secretary, Guy's Hospital Medical School, London Bridge, SE1 9RT, quoting Ref. M.C.1. 1410(A)

FELLOWSHIPS IN ENVIRONMENTAL RESEARCH

The Monitoring and Assessment Research Centre at Chelsea College, University of London is carrying out a research programme on pollution assessment.

The broad objectives of the programme are to improve understanding of the movement and effects of pollutants in the environment so that harm to man and his resources from pollution can be estimated and assessed.

The work is sponsored by the United Nations Environment Programme and The Rockefeller Foundation and has the support of the Scientific Committee on Problems of the Environment.

Applications are invited for senior visiting research fellowships from scientists who could make contributions in one of the following work areas:

1. The development and use of environmental transport models, along various critical pathways, which will enable the prediction of total exposure of man and other important targets to certain pollutants.
2. The evaluation of the effects of environmental pollution in particular to
 - * review current approaches to determine dose-response relationships for humans and other biota and the toxicological and epidemiological information required;
 - * use dose-response relationships to define exposure limits;
 - * develop methods of assessing the harm to intact ecosystems resulting from environmental stress.

In the first instance, appointments will be made for a period of one to six months. These fellowships are particularly suited to applicants wishing to come on sabbatical leave or on a similar basis. Financial support will be considered by M.A.R.C. on a case by case basis but may include contributions towards travel expenses as well as a *per diem* allowance during the term of the appointment.

Potential applicants may obtain further information from the Director, Monitoring and Assessment Research Centre, The Octagon Building, 459A Fulham Road, London SW10 0QX to whom applications together with *curriculum vitae* and the names of two referees should be sent. (1390)A

KING'S COLLEGE HOSPITAL MEDICAL SCHOOL

(University of London)

Denmark Hill

London SE5 8RX

DEPARTMENT OF OBSTETRICS AND GYNAECOLOGY

Applications are invited from graduates or suitably qualified people for the post of Research Technician on a project for which experience of at least one year in tissue culture and cytogenetics is essential. In addition, histological and histochemical techniques will be applied in this area of pre-natal diagnosis of inherited diseases.

Starting salary £3,112 inclusive.

For informal enquiries please contact Dr J. Singer on 01-274 6222 extension 2349. Applications in writing giving full details of qualifications and experience should be sent to the Secretary of the Medical School at the above address. Closing date March 2, 1978. 1464(A)

RESEARCH ASSISTANT IN ATMOSPHERIC SCIENCE (AERONOMY)

The Department of Atmospheric and Oceanic Science of the University of Michigan has a postdoctoral research position available in the area of terrestrial and planetary aeronomy. This position involves analysis and interpretation of data obtained from a variety of space missions (e.g. Pioneer-Venus, Voyager, Dynamics Explorer, etc.). This work is to be carried out in collaboration with Professors T. M. Donahue and A. F. Nagy. Send resumes to: University of Michigan, Office of Professional and Administrative Staff Services, 1020 "SN" L.S. and A. Building, Ann Arbor, Michigan 48109. A non-discriminatory, affirmative action employer. 1441(A)

YALE UNIVERSITY SCHOOL OF MEDICINE NEUROBIOLOGIST

The Department of Physiology is seeking a neurobiologist at the Assistant Professor level. Preference will be given to individuals whose work relates to the function of the central nervous system. The position permits considerable time for research; opportunities exist for graduate and medical student teaching.

Please send curriculum vitae, bibliography and the names and addresses of three references to:

CHAIRMAN
Neurobiologist Search Committee
Department of Physiology
Yale University School of Medicine
333 Cedar Street
New Haven, Conn. 06510

Applications from women and members of minority groups are encouraged. An Equal Opportunity/Affirmative Action Employer.

Applications should be received before March 30, 1978. 1419(A)

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Cedarlane Laboratories Limited,
493A Wellington Road South,
London, Ontario,
CANADA N6C 4R3 1443(A)

UNIVERSITY OF BIRMINGHAM

DEPARTMENT OF ANATOMY

Applications are invited from graduates in medicine, medical or biological science for the post of LECTURER in the Department of Anatomy (Professor J. J. T. Owen). The Department has broad research interests but preference will be given to candidates with research interests in the field of immunobiology. Training in the teaching programme of the Department will be given if necessary, and previous experience is not essential.

Salary on the scale £3,333 to £6,655 plus superannuation.

Further particulars about the post can be obtained from Professor Owen. Applications should be sent to the Assistant Registrar, the Medical School, Birmingham B15 2TJ, giving the names of three referees, by March 17, 1978. 1426(A)

CENTRAL BIRMINGHAM HEALTH DISTRICT THE CHILDREN'S HOSPITAL, TECHNICIAN or BIOLOGICAL SCIENCE GRADUATE

required for a research post. The successful applicant will be concerned with a leukaemia and non-Hodgkins Lymphoma study. Previous experience in immunology helpful but not essential. Appointment for one year in the first instance.

Salary according to experience and qualifications.

For further details and an application form please contact Dr F. G. H. Hill, Consultant Haematologist, Haematology Department, The Children's Hospital, Ladywood Middleway, Birmingham B16 8ET. Telephone: 021-454 4851. 1402(A)

TECHNICIAN, H.N.C. or science graduate, to help with a grant financed project concerning antibodies in tumour immunology at the Institute of Cancer Research, Sutton, Surrey. Knowledge of protein chemistry and immunological techniques would be an advantage.

Salary in scale £2,982 to £4,258 p.a. plus London Allowance of £354 p.a., depending on qualifications and experience.

Applications in duplicate with the names of two referees to the Secretary, Institute of Cancer Research, 34 Sumner Place, London SW7 3NU, quoting ref. 301/B/40. 1438(A)

HERIOT-WATT UNIVERSITY DEPARTMENT OF ELECTRICAL AND ELECTRONIC ENGINEERING SCANNING ELECTRON MICROSCOPY X-RAY ANALYSIS Applications are invited for an EXPERIMENTAL OFFICER

to operate a scanning electron microscope with an X-ray analyser attachment.

Applicants should have supporting experience in related fields such as electronics, photography and data analysis. The Department provides a service for research, on dielectric, metallurgical, geological and biological materials, much of it in connection with the Heriot-Watt/S.R.C. Centre for Marine Technology. The Experimental Officer will be responsible for achieving high quality results for such research but will be encouraged to develop his own research interests.

Salary scale: Research Staff—Range 1A £3,333 to £5,626. (Salary scales currently under negotiation).

Please apply in writing for an application form to the Staff Officer, Heriot-Watt University, Chambers Street, Edinburgh EH1 1HX. 1440(A)

South African Astronomical Observatory Research Posts

Two temporary (3 year) or permanent research posts are available at the South African Astronomical Observatory which is financed jointly by the Council for Scientific and Industrial Research (South Africa) and the Science Research Council (United Kingdom). The astronomy staff of the SAAO is based in Cape Town where the main offices, etc. are situated. Most observing facilities are at Sutherland (Cape Province) where the SAAO has 1.9m; 1.0m; 0.75m and 0.5m reflectors equipped with a range of modern instrumentation.

Preference will be given to applicants with research experience and a Ph.D. degree or equivalent, and whose main interest is observational astronomy.

Salary will be determined by qualifications and experience. Assistance will be given with transfer expenses.

Fringe benefits include 39 days leave per annum (maximum), generous sick leave, and medical and pension schemes.

Enquiries should be directed to the Office of the Scientific Counsellor,

South African Embassy:

278 High Holborn, London WC1V 7HE (Tel. 01-242 1766)

59, quai d'Orsay 75007 Paris (Tel. 555-92-37)

P.O. Box 12-1366 Tehran (Tel. 62-1835/6/7)

5300 Bonn 2, Auf der Hostert 3 (Tel. 36 3047/48)

3rd Floor, 2555 M Street N.W. Washington D.C. 20037

(Tel. 833-3860)

1453(A)

THE LONDON HOSPITAL MEDICAL COLLEGE (University of London) BONE AND JOINT RESEARCH UNIT

RESEARCH ASSISTANT (IMMUNOCHEMIST)

Applications are invited for the post of Research Assistant, from graduates with a suitable qualification, to assist in research on human protein immunochromatography. Further, an interest in cellular immunology will be an asset. This post, which is for a limited duration, is financed by a Grant from the M.R.C. to Dr E. J. Holborow from whom further details may be obtained, telephone no. 01-247 5454 Ext. 420. Initial salary will be within the range £2,904 to £3,761 (under revision) plus £450 London Allowance and superannuation under the U.S.S./N.H.S.

Written applications (quoting reference RA/IM/2/78) to The Secretary, The London Hospital Medical College, Turner Street, London E1 2AD within 21 days. 1452(A)

UNIVERSITY OF LONDON KING'S COLLEGE DEPARTMENT OF BIOCHEMISTRY CHIEF TECHNICIAN Grade 7

Applications are invited from appropriately qualified (at least H.N.C.) technicians with at least 10-12 years experience for this responsible post. Preferred age 30-40. The successful applicant will be in charge of the organisation and supervision of all technical services under the general direction of the Head of Department.

Salary on scale £4,719 p.a. rising to £5,242 p.a. inclusive. Application form and further particulars from: The Head Clerk, Ref. 191123/N, King's College London, Strand WC2R 2LS. 1423(A)

GLASSBLOWER required for construction and repair of chemical glassware. Write giving details of experience: Maybridge Chemical Co. Ltd. Tintagel, N. Cornwall. 1421(A)

ST JUDE CHILDREN'S RESEARCH HOSPITAL

Fellowships available July 1, 1979 at St Jude Children's Research Hospital, Memphis, TN for physicians or basic scientists: David A. Karnofsky Fellowship for training beyond postdoctoral level in cancer research and Leon J. Journey Fellowship for postdoctoral training in biomedical research.

Areas of training include: biochemistry, hematology-oncology, immunology, infectious diseases, nutrition and metabolism, pathology, pharmacology, radiology, surgery and virology.

Stipends: \$12,000 to \$18,000. Open to international competition. September 1, 1978 deadline. Write: Medical Director, St Jude Children's Research Hospital, P.O. Box 318, Memphis, Tennessee 38101. 1425(A)

THE UNIVERSITY OF SHEFFIELD

DEPARTMENT OF PHYSIOLOGY

Applications are invited from men and women for a post of RESEARCH ASSISTANT in the above Department tenable as soon as possible for up to three years. The successful applicant would be required to join a group working in one of the following areas: Central nervous control of gastrointestinal function.

Secretory mechanisms of exocrine glands.

Other areas of interest would, however, be considered. Initial salary in range £2,904 to £3,547 a year on the R&A Range IB scale rising to £4,190 a year. Particulars from the Registrar and Secretary, the University, Sheffield S10 2TN to whom applications (5 copies), should be sent by March 3, 1978. Quote Ref. R.73/G. 1400(A)

THE MIDDLESEX HOSPITAL MEDICAL SCHOOL (University of London)

BIOPHYSICAL ENDOCRINOLOGY UNIT, DEPARTMENT OF PHYSICS AS APPLIED TO MEDICINE

Applications are invited for a Research Assistant 1A; starting salary, depending on age and qualifications, of £3,783 to £4,212 including £450 per annum London Allowance.

Suitable for a postdoctorate, this post will be available for two years, funded by a Medical Research Council Programme Grant. The project will involve the in vitro assay of pressor substances including the preparation and use of dispersed muscle cells. Preparation and investigation of other biological cells may be involved.

Applications, together with the names of two referees should be sent to the Secretary, Department of Physics as Applied to Medicine, Windeyer Building, Cleveland Street, London W1P 6DB. 1462(A)

ST GEORGE'S HOSPITAL MEDICAL SCHOOL (University of London) TECHNICIAN

required to assist with laboratory research on the physiology of animals including the nervous system and studies on the secretion of mucus. Experience desirable, but not essential since training in the various techniques will be provided. The grading of the post (up to a maximum of University grade 2B £3,155 p.a.) will be determined according to qualifications and/or experience. Application forms available from the Establishment Officer (Ref. Phys), St George's Hospital Medical School, Cranmer Terrace, London SW17 0RE. 1459(A)

ST THOMAS' HOSPITAL MEDICAL SCHOOL (University of London) London SE1 7EH

APPLIED NEUROPHYSIOLOGY

A Research Fellowship provided by the Medelec Co. Ltd. is available at the Sherrington School of Physiology and the Department of Rheumatology (Electrodiagnosis), St Thomas' Hospital, London SE1 7EH. Medical graduate to be appointed for 2 to 3 years, should have some experience of electrodiagnosis. Enquiries to Professor A. Taylor, Sherrington School.

1458(A)

FELLOWSHIPS

ROYAL POSTGRADUATE MEDICAL SCHOOL POSTDOCTORAL BIOCHEMIST/ PHARMACOLOGIST

Applications are invited for an M.R.C. Postdoctoral Research Fellowship, tenable for up to three years, for studies on aspects of the role of prostaglandins in human disease. Candidates should possess a Ph.D. in Biochemistry, Pharmacology or related subject and should have experience in (or a keen interest in learning) those analytical techniques, e.g. gas chromatography-mass spectrometry, employed in the determination of trace components in biological fluids.

Salary up to £4,256 per annum inclusive.

Applications, enclosing a curriculum vitae and the names and addresses of two referees, should be sent as soon as possible to the Personnel Officer, R.P.M.S., 150 Du Cane Road, London W12 0HS, quoting ref. no. 20/466/N. 1463(E)

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Travelling Fellowships

Each year we have £1,500 available for travelling fellowships to be awarded to scientists or journalists who want to travel on *Nature's* behalf to parts of the world where we do not as yet have strong editorial contacts.

Sabbaticals

There are occasional opportunities for scientists to spend a period of a month or two in the *Nature* office working alongside our editorial staff.

We are at present inviting applicants for these positions for the coming year. Further details and application forms are available from The Editor, *Nature*, 4 Little Essex Street, London WC2. 1429(E)

FELLOWSHIPS—continued

UNIVERSITY OF
NOTTINGHAM
SCHOOL OF AGRICULTURE
POSTDOCTORAL
FELLOWSHIP IN
REPRODUCTIVE
PHYSIOLOGY

Applications are invited for a post-doctoral fellowship to join a research team investigating problems of reproduction in lactating dairy cows. The project involves liaison with dairy farmers and veterinary surgeons in studies on the causes and incidence of bovine subfertility. A knowledge of radioimmunoassay procedures an advantage.

Applications, giving the names of three referees together with a curriculum vitae to be submitted to Prof. G. E. Lamming, University of Nottingham School of Agriculture, Sutton Bonington, Nr. Loughborough, Leics. by March 1, 1978. 1451(F)

RESEARCH FELLOWSHIP
Imperial Cancer Research Fund
Laboratories, Mill Hill, NW7

A postdoctoral Fellow is required to join a group working on the genetic and biochemical analysis of certain heat activated loci in *Drosophila melanogaster*, using cloned DNA fragments to investigate their organisation and expression.

Appointment will be for two years in the first instance with possible extension for a third year. Salary with entry according to qualifications and experience within range £4,649 to £5,669.

Further information from Dr D. Ish-Horowitz (01-959 3236). Applications with curriculum vitae and names of two referees, should be sent to The Secretary, Imperial Cancer Research Fund, Lincoln's Inn Fields, London WC2A 3PX by March 17, 1978. 1475(E)

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SYMPOSIUM

SYMPOSIUM

on

"THEORY AND PRACTICE
IN AFFINITY TECHNIQUES"

APRIL 10-12, 1978

The workshop will be held at the Max-Planck-Institut für experimentelle Medizin, Göttingen, West Germany.

Lectures and Round Table Discussion will be on special features in Affinity Chromatography (recent developments) and Affinity labelling of enzymes, receptors and hormones, Radioimmunoassay, Enzyme Immunoassay.

Speakers will include:

C. R. Lowe (U.K.), F. von der Haar (Germany), P. D. G. Dean (U.K.), R. L. Easterday (USA), J. R. Coggins (U.K.), R. Abeles (USA), H. Fasold (Germany), F. C. Hartman (USA), M. Wilchek (Israel), E. Kuchler (Austria), D. G. Knorre (USSR), A. Maclieck (Germany), R. Leute (USA), Lequin (Netherlands).

Participation:

Applications should reach Dr P. V. Sundaram, Max-Planck-Institut für experimentelle Medizin, D-3400 Göttingen, Hermann-Rein-Straße 3, West Germany, before March 20, 1978, declaring research interests. A registration fee of DM 150, will be charged. Hotel arrangements will be left to participants but hotel list supplied. Participants will be responsible for their travel and living expenses.

Organizers:

Dr P. V. Sundaram and Professor F. Eckstein
(Telephone: 0551/303 274). 1444(M)

STUDENTSIPS

UNIVERSITY OF
ABERDEEN
FACULTY OF MEDICINE
RESEARCH
STUDENTSIPS

Applications are invited from recent first or upper second class honours graduates in a biological science subject, and from those who expect so to graduate in 1978, for research studentships established by the Faculty of Medicine from its Medical Endowment Funds. Successful applicants will study for the degree of Ph.D. on an approved subject in one of the Departments of Biochemistry, Developmental Biology, Pathology, Pharmacology or Physiology, commencing in October 1978.

The value of the studentship is £1,475 p.a. (for students living away from home) with payment of tuition fees and other allowances.

Application forms and further particulars of the projects may be obtained from D. K. Yule, University Office, Regent Walk, Aberdeen AB9 1FX to whom applications should be submitted by March 17, 1978. 1468(F)

THE WEST OF SCOTLAND
AGRICULTURAL COLLEGE
Crichton Royal Farm,
Dumfries

RESEARCH STUDENTSHIP

Applications are invited for Research Studentship at the above experimental husbandry farm, to study herbage production and its utilisation by dairy cattle. The current value of the award, which will be tenable for up to three years, is £1,478 p.a. plus fees and approved travel expenses.

Applicants should have a first or second class honours degree in agriculture or agricultural science and the successful applicant will be required to register for a higher degree.

Further details may be obtained from the Secretary, The West of Scotland Agricultural College, Auchincruive, Ayre KA6 5HW, with whom applications should be lodged by March 17 1978. 1457(F)

COURSES

F.E.B.S. ADVANCED COURSE No. 54

MITOCHONDRIAL BIOGENESIS IN
ANIMAL DEVELOPMENT

JUNE 10-25, 1978, KOTOR, YUGOSLAVIA

INSTITUTE FOR BIOLOGICAL AND MEDICAL SCIENCES
MONTENEGRO,

UNIVERSITY OF TITOGRAĐ (YUGOSLAVIA)

INSTITUTE OF BIOLOGICAL CHEMISTRY AND

CENTRO C.N.R. STUDIO SUI MITOCONDRI

E METABOLISMO ENERGETICO

UNIVERSITY OF BARI (ITALY)

The experimental program is as follows:

- Isolation of mitochondria from animal cells
- Isolation of mitochondrial DNA
- Endonuclease cleavage map of mitochondrial DNA
- Cloning of mitochondrial DNA fragments
- Extraction and characterisation of mitochondrial components
- Mitochondrial macromolecular synthesis during early developmental stages
- Nuclear control on mitochondrial biogenesis: anucleation and related techniques.

The speakers will include:

G. Bernardi (Paris), H. Bresch (Hannover), R. Butow (Dallas), G. Giudice (Palermo), V. Glišin, (Kotor), A. M. Kroon (Groningen), E. Quagliariello (Bari), C. Saccone (Bari), H. Weiss (Heidelberg).

The reaching staff will include:

N. Moškov, V. Deretić, M. Solomun (Kotor), M. Greco, G. Pepe, G. Del Prete (Bari), H. Bakker (Groningen), A. Nicotri (Roma), A. M. Rinaldi (Palermo).

The language of the course is English. Attendance at the course will be limited to 20 participants. Preference will be given to applicants from the European area at the postdoctoral level with research experience who plan to engage in investigations of the role and function of Mitochondrial Biogenesis in animal cells.

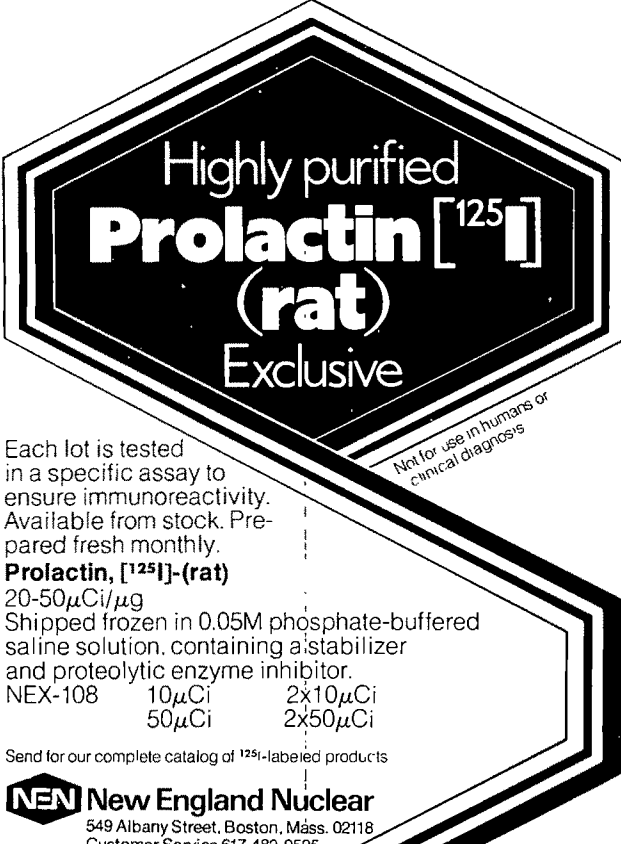
The participation fee will be 125,000 It. lire or 2,900 Yu. dinars.

The participants are expected to pay their own travel. The food and lodging expenses will be covered by the organisers. To successful applicants few accommodation fellowships will be awarded.

Applications with short curriculum vitae and any information which might help in the evaluation of the application should reach by April 15: Dr Vladimir Glišin, P.O. Box 80, Kotor, Yugoslavia 81330 and/or Dr Cecilia Saccone, Istituto di Chimica Biologica, Via Amendola 165/A, Bari (Italy).

ORGANISERS: Vladimir Glišin, Ernesto Quagliariello and Cecilia Saccone

OTHER SPONSORSHIPS: E.M.B.O., C.N.R. (Italy), University of Titograd (Yugoslavia), University of Bari (Italy). 1474(D)



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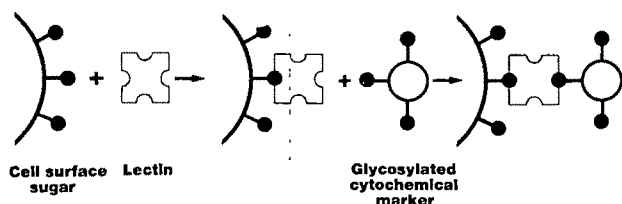
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4549	Fucosyl-Ferritin : Fuc-Fer	U.e.A-F, L.t.A
4550	Mannosyl-Ferritin : Man-Fer	CON-A
4551	Glycopeptidyl-Ferritin : T.G.-Fer	CON-A, PHA-E Ls, S.B.A., P.N.A.

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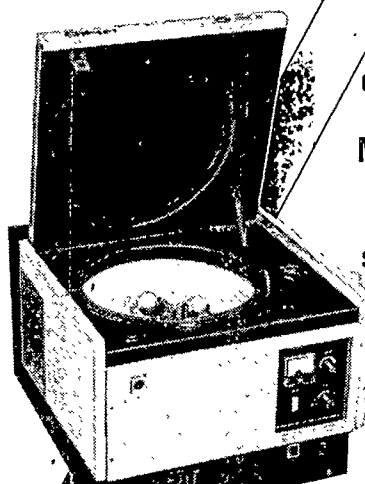
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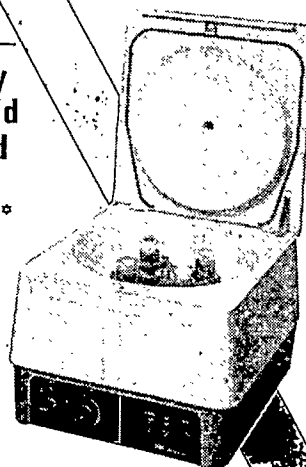


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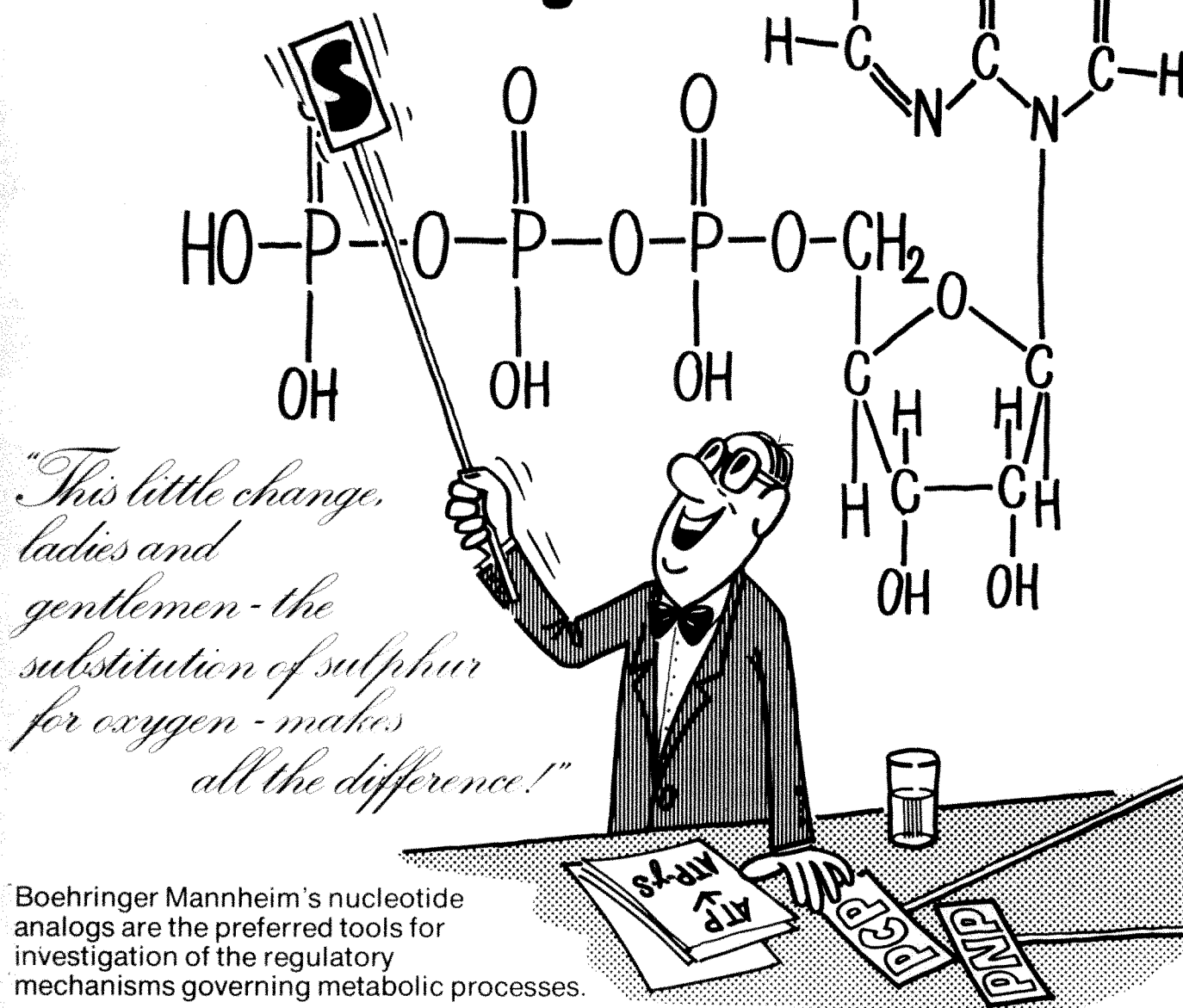
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Published weekly
ISSN 0028-0836

Registered as a newspaper at the
British Post Office

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4 Little Essex Street, WC2R 3LF
Telephone: (01) 836 6633 Telex: 262024
Telegrams: Phusis London WC2R 3LF

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1976 Index (post-paid) U.K. £2.50. Rest of
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Regenerated axolotl limb.
See pages 705 and 760.

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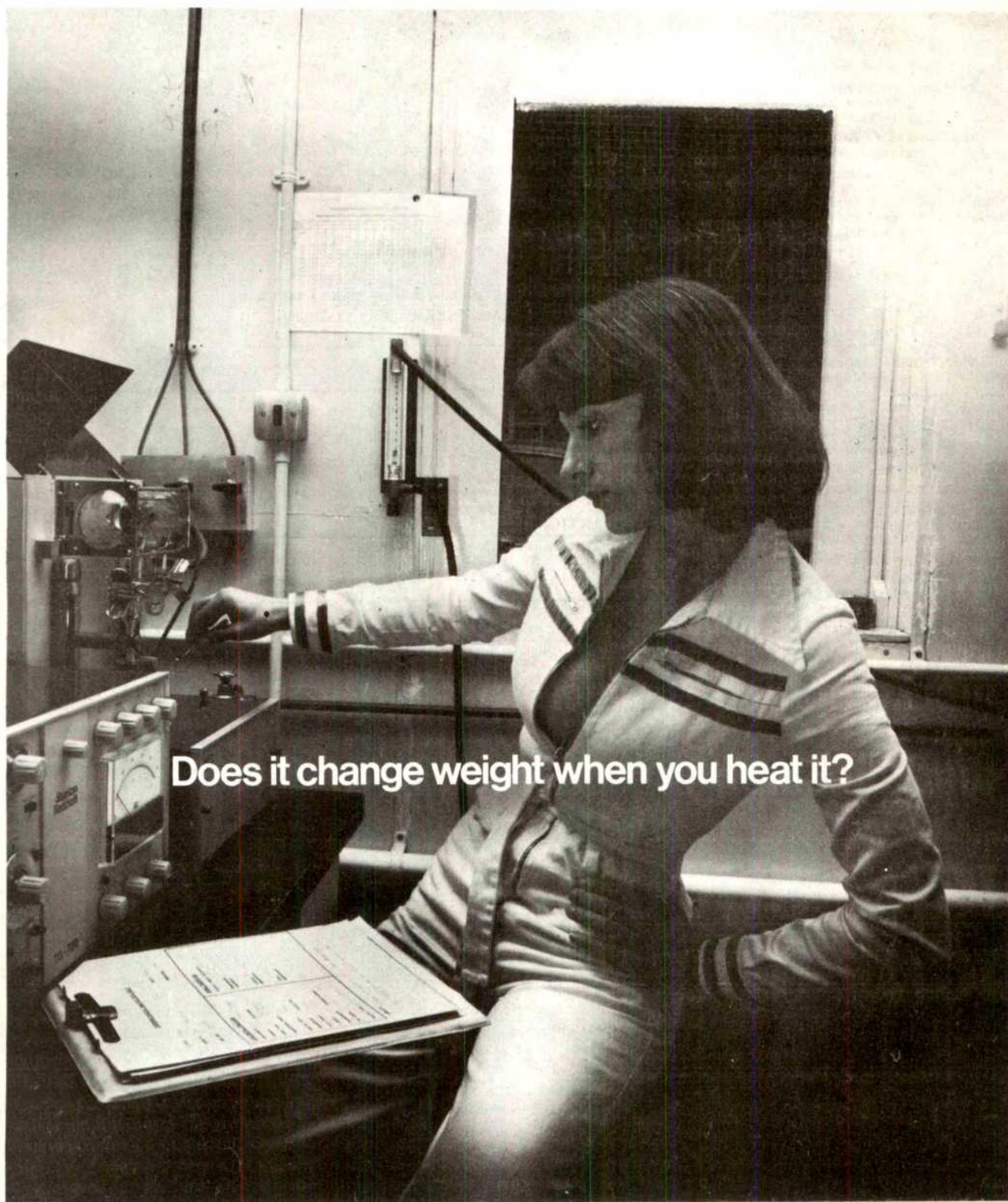
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Corrigendum

In the letter by P. E. Thorpe *et al* on p.752 of this issue, in Fig. 2 (a) it represents results from 1-h incubation with toxin (not 24h) and (b) represents its values from 24-h incubation.

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23 February 1978

Coping with energy's nasty surprises

WHATEVER one may say about the general opacity of Whitehall and the British decision-making machine, Mr Tony Benn, Secretary of State for Energy, has ensured that his department is immune from much of the criticism. The proliferation of advisory councils, working groups, discussion documents and public meetings certainly means that Mr Benn shares his problems with the public-at-large; it probably also means that the decisions he makes are more enlightened, but whether those decisions turn out right in the long run is, in matters of energy, largely in the hands of people and events remote from Britain and British control. Thus it is reassuring that the one word which keeps cropping up in the Department of Energy's new consultative document *Energy Policy* (HMSO, £2.15) is 'flexibility'.

The Green Paper, although generated within the department, has also been discussed by the recently-formed Energy Commission, a body representing workers and management in the energy industry, and conservation, environment and consumer interests. Having been seen by many pairs of eyes both within the department and beyond it, the paper contains much middle-of-the-road common-sense, little that will surprise and a general commitment to keeping options open as long as possible. For instance, on nuclear strategy, "there is a need to have a capability to expand nuclear power rapidly in the late 1980s and 1990s if that course proves to be economically desirable and acceptable in other respects". Who would disagree with that?

Yet in spite of the genial optimism that generally surrounds the first major policy document to emerge from the department since North Sea oil flowed in any great quantity, there are problem areas; gas prices are perhaps the most serious. Natural gas is a relatively cheap resource which, since 1960, has progressively replaced coal as the domestic sector's most popular fuel; parenthetically, the supply of heat to the domestic sector has barely changed in 25 years, although the amount of useful heat consumed has risen by 75% with the switch away from coal and towards central heating. But the very cheapness of gas has acted, claims the department, against the best interests of a broadly based energy policy by prejudicing long term growth plans in the coal, electric and nuclear industries. So one of the decisions that has to be taken in the next two years or so, according to the Green Paper, is energy price structure, meaning how much the cost of gas should go up. Other short-term decisions with long-term implications are

- the fast reactor; UKAEA are bound soon to come up with a proposal for a demonstration commercial fast reactor which will have to go first to a public inquiry,
- whether a pipeline should be built to collect the heavier gas generally associated with North Sea oilfields,

- how much tougher conservation measures can be made, and
- the shape of future R & D, most notably where major efforts should be made in alternative energy sources.

Enthusiasts for alternative energy sources will be disappointed by the tone of the Green Paper. There are few concessions to 'softer' energy paths, and by the year 2000 alternative energy sources such as solar, waves, wind, tides and geothermal are seen as contributing a total of only 2%, if that, to the nation's needs. The recently installed Chief Scientist, Sir Hermann Bondi, is said to be looking right now at R & D strategy and it is to be hoped that he will put a little more optimism into these figures.

The one thing that the document does not do is discuss the possible nasty surprises which could cause a major derailment of the government's energy policy. Of course flexibility is meant to minimise the impact of nasty surprises but several could be thought of with disagreeable consequences. Two in particular might be mentioned. First, planning procedures have been getting distinctly more complex in recent years and there have been moves to make enquiries much wider-ranging than ever before. On major developments it is no longer simply a question of whether the local neighbourhood is going to be much inconvenienced—much broader policy questions are now being asked. Is it possible that a succession of questions, such as the opening up of new coalfields, should be decided against the government's intentions? If so, planning procedures could play havoc with a balanced energy policy. Second, the question of carbon dioxide and atmospheric warming seems by no means resolved. There is no doubt that the concentration is rising at present by more than 2% every ten years, and that there is no way of reversing this trend in the foreseeable future. In little more than fifty years' time, in some projections, the carbon dioxide concentration could be rising much more dramatically and be approaching double its present value.

There is no clear evidence today for a global warming from this cause, but, for all the uncertainties associated with climatic modelling, a doubling of the concentration is unlikely to occur without perceptible change. A warming of a degree or so, such as is often predicted, might be an agreeable bonus in temperate climates, but the real question mark is over the behaviour of the West Antarctic Ice Sheet (*Nature* 271, 321 (1978)). If this started to break up it would be far too late to prevent a catastrophic rise in sea level, so pressures for worldwide restraint in fossil fuel usage must come sooner rather than later. CO₂ might have as disturbing an influence on energy policy in the 1980s as did OPEC in the 1970s. □

Research in Greece

In this second article on Greek science, E M Pantelouris, assesses the effort put into non-university research.

MORE active Greek researchers work abroad than inside Greece. The recent death of one notable Greek scientist, K. Kotzias, who pioneered the treatment of Parkinson's disease with L-Dopa in the United States, only serves to emphasise this fact. And it was another Greek, George Papanicolaou, also working in the States before Kotzias' time, who first established the science of exfoliative diagnostic cytology.

Scientific papers, however, do come out of Greece. A computerised data search of the *Science Citation Index*—which may well cover 90% of the world's original research work—picked some 2,000 papers published in 1974-77 which are attributed to research centres in Greece (see table). Although there must be limitations to drawing conclusions from data about the quantity of published items, some general points can be made. In this article, such data will be taken as starting points for a general look at non-university research.

Non-university research

Pride of place in non-university research belongs to 'Demokritos', a research centre for fundamental science in Athens. The number of publications attributable to its 40 biological researchers equals the number attributable to equivalent researchers in Athens University; and its 140 researchers in physics and chemistry produce more publications than their university colleagues.

Scientists at Demokritos are, of course, free from teaching commitments; but so are scientists at other less effective institutes. The factors contributing to Demokritos' success seem to be the lack of the narrow compartmentalisation of the university, the relative independence of scientists in planning their research and competing for project grants, the higher qualifications possessed by practically all scientific staff, and good facilities.

In addition to fundamental research, the centre tackles some practical tasks. These include the production of 130,000 μCi of short-lived radio-isotopes (another 35,000 are imported) for hospital use (the centre was in fact founded around the reactor and still carries the name 'nuclear research centre'); dealing with methods for searching for uranium deposits; main-

taining a human tissue bank for transplants; and doing fundamental work on photosynthesis with the use, for biological control, of sterilised males of the insect pest of the olive, *Dacus oleae*. Demokritos would probably be a good place for postgraduate students to work.

The papers from 'other centres' come from a variety of establishments, including the National Research Foundation (with 30 publications) industrial laboratories and so on. For convenience, publications from the small, young universities of Yannina and Patras are included in this category.

Medical research

The medical faculty of the University of Athens has recently produced, with some pride, a list of 5,859 publications by its members in 1970-75. The press, however, soon pointed out that of these, the bulk are locally published articles, case discussions and so on. Only 757 are refereed papers in international journals; and some of those cover work carried out abroad. The *Science Citation Index* figure of 479 (see table) is limited to the years 1974-77 and does not seem to contradict this figure.

It cannot be disputed, however, that hospital doctors come across ample case material, and have sufficient facilities for doing clinical tests, leading to publishable observations; the stimulus, and in fact competition, for publishing such observations is undoubtedly strong. But few papers deal with biomedical, as distinct from clinical, research. Some medical research teams have become internationally known for a continuing body of work in certain fields, such as the

locally important problems of haemoglobinopathies and of enzyme polymorphisms.

But the prestige of the Athens medical faculty is not founded only on the number of papers it produces, but also on the social impact of the medical profession. Has the profession been able to provide adequate medical cover for the population? Is the standard of medical care much higher in the lucrative private sector than in the public sector? Is the profession avoiding the worst excesses of private practice?

These are legitimate questions when thousands of patients travel every year to seek treatment abroad, especially as they cannot help but compare the ethical attitudes and fees in other countries with those back home. It would also be interesting to find out whether the lucrative character of private medicine in Greece—or more accurately, of a special circuit of private medicine including a high proportion of university professors—will attract to Athens, when Greece enters the European Economic Community many doctors from other member countries.

Agricultural research

Another sector where social impact is important is agricultural research. The achievements of numerous stations and institutes run by the Ministry of Agriculture are not adequately reflected in the table because much of their work ends in filing cabinets, or is translated into circulars; and work lasting ten or more years to establish a new variety of wheat may yield very few papers.

But it may yield a lot of grain. In the 1920s when one and a half million refugees, mainly from Ionia, arrived in Greece destitute, the average wheat yield was 220 kg per acre. The late Papadakis, a dedicated agriculturist, started work at a small station in Thessaly with the aim of breeding new varieties better adapted to the country. It is largely thanks to the breeding work he initiated that, today, Greece

Numbers of papers listed in the Science Citation Index 1974-77 as originating from Greece.

Institution	Medicine	Life sciences	Science and Engineering
University of Athens	479	66	142
'Polytechnion' of Athens			96
University of Thessaloniki	57	66	133
Agricultural Research Stations and Institutes		29	
Demokritos	13	68	228
Other medical centres	297		
National Research Foundation			30
Other science laboratories			194
Other life science laboratories		46	
	846	275	823
Total:		1944	

Dr E. M. Pantelouris visited Greece as a Nature travelling fellow

is self-sufficient in wheat, with an average yield of 1,000 kg per acre, and could, if it were economical, produce some wheat for export. Greece might in fact do that specifically for the hard wheat required by EEC industries.

From that Cereal Improvement Station of Thessaly has developed the Cereal Breeding Institute of today at Thessaloniki; and the country has also become self-sufficient in barley for animal feed, and in rice. Corn is a problem, mainly because in the Greek climate it requires irrigation, and the country has very little irrigated land. All the work on cereals is carried out by about 20 members of staff at the institute, with the help of other people at peripheral testing centres. This is cost-effective research.

The same can be said about other research stations dealing either with single crops (such as cotton, in which again self-sufficiency was achieved years ago) or with livestock. The Yanitsa Livestock Improvement Station has had successes of great potential with crosses of the local Chios strain of sheep with Friesland sheep.

There are also some larger, interdisciplinary, institutes. The Benaki Phytopathological Institute near Athens deals mainly with entomology. Although founded in 1929 as an independent establishment, it is now largely supported by the Ministry of Agriculture. It deals with problems such as the notorious *Phylloxera*, which is reappearing in the vineyards of Crete, and the biological control of citrus-fruit scale insects.

The two forest institutes, at Athens and Thessaloniki, are also multidisciplinary. Forests in Greece are largely state owned, and are run by a system where the forestry service supervises all operations in co-operation with local villagers on contract. The institutes therefore have a variety of concerns, ranging from timber technology, mechanisation, and the introduction of new economic plantations, such as poplar and eucalyptus, to the protection of forests from fires, which caused enormous damage last summer. The forest institutes could perhaps concentrate more on forest and soil preservation in the least productive and uneconomic areas.

The work of these stations and centres suggests that small research groups, geographic isolation, and restricted facilities are not necessarily disadvantages so far as applied research of this type is concerned. Success hinges more on leadership and dedication. Applied research may well benefit more generally from the work of small task-oriented groups. Fundamental research on the other hand would be



Karamanlis: implementing a new science policy

better served in larger, multidisciplinary centres.

A New Research Policy

The Greek government is taking measures to stimulate 'relevant' research.

Greek agriculture suffers because of the small acreage per farmer, and can only be uplifted by technological progress and, in the last resort, by conversion to irrigation. Industry has, for some years now, exceeded agriculture in importance. To make both viable within the EEC, and to raise the per capita income, which now hovers around the level of Ireland, the government believe that indigenous activity in the fields of applied R&D is essential.

In general terms, the following policy has been formulated. On-going research will continue to be supported at present levels. New funds, however, will be made available specifically for research related to a few key requirements of the country's development plan. These funds will not be dispensed as support grants for institutions as such, but in the form of grants for specific research projects, submitted by individual or grouped workers. Foreign scientists are not excluded.

A law institutionalising the policy was passed last year and the Prime Minister, Mr Karamanlis, is by all accounts pressing for a speedy implementation. A cabinet committee is in control, and a special civil service department has been set up at the Ministry of Coordination, headed by Dr Argyropoulos, an electronics expert returned from the United States. A census of laboratories has been carried out, but due to lack of cooperation, it is as yet incomplete.

The new policy combines two pragmatic approaches: that of letting work now in progress continue; and of setting up target-oriented research.

One of the potential virtues of the combination is that it might forestall the reappearance of the old pattern in the guise of the new.

Success will now depend on how the policy is implemented. Firstly, an advisory council will be appointed by the government. Its appointment will be crucial because it must be efficient and imaginative, and must inspire complete confidence in the research community. The second crucial task is the definition of a handful of research sectors where efforts will be concentrated. If the definition is too narrow and specific it may inhibit creative processes. If too wide, it may be operationally meaningless, and provide no guidance.

For example, one problem Greece has to face is that of soil erosion. Limestone covers one third of the country, mostly in the form of mountains where the extremely fragile ecosystem has been degraded by destruction of forest and by erosion to the state of sparse maquis vegetation and small pockets of soil between exposed rock. What is not already lost must be preserved and every effort made to recoup as much as possible. This will involve preventing the winter rain from causing destructive floods and damaging agriculture and even dwellings.

Defined in this way, the problem appears to be one of traditional torrent regulation so that the seasonal rain is helped to flow safely away. But the water is a precious asset, and the key to making economically viable the Greek farmer's small acreage must be to conserve the winter rainwater for use in the summer. The problem may now be redefined as one of storing the winter rain in each catchment area. Perhaps it could be channeled to underground cisterns, or at least retained in soil 'sponges' created at the outlet of every torrent and gully. Perhaps chemical films should be used to reduce evaporation from reservoirs and special tubing systems (there is one under study by a Greek plastics firm) might permit the speedy and economic irrigation of crops and trees. Perhaps there might even be physico-chemical methods for producing a thin layer of soil on the limestone surface; and perhaps arid-zone plants should be seeded there to retain and improve the soil so that a future generation might reafforest it.

The definition of priority areas must be comprehensive enough to evoke all approaches which, when combined might lead to a solution—a definition of targets and problems, not just of projects and programmes, yet not vague and platitudinous. The successful implementation of the new policy is bound to be a difficult undertaking. □

AAAS

The expert has no clothes

WASHINGTON is a destroyer of myths. It destroyed Richard Nixon. It is destroying Jimmy Carter. And last week, at the annual meeting of the American Association for the Advancement of Science, it was happily destroying the myth of the expert. In the climate prevailing at the meeting, any of the 5,500 scientists attending who were unconscious of or unconcerned by the political, social, or psychological dimensions of their work were made to feel distinctly uncomfortable. The conclusion: scientists must absorb, welcome, and understand politics if they are to retain a niche in the ecology of the latter quarter of the 20th century, and not attempt to ignore or deflect politics through the exercise of defensive public relations.

Six sessions addressed such issues: in sociobiology, where the validity of human sociobiology is a political matter as much as a scientific one; in recombinant DNA, where the assessment of risks is still a political issue, complicated now by the introduction of firms such as Genentech Inc. which hopes to produce insulin by the end of the year, and is not controlled by the NIH guidelines (neither are any firms, defence establishments, or the CIA); in the protection of 'whistleblowers'—people who, like Dr Thomas Mancuso, working for a government agency, call the wrath of the system upon them by claiming a danger exists that the agency management would prefer to ignore (Mancuso claimed that workers in the Hanford nuclear plant, Washington State, were subject to a 5% increased cancer risk, and was taken off the study); in the reception of unconventional science, where people with wild but possibly fruitful ideas receive short shrift from the scientific establishment without much evidence of proper assessment; and in the reproducibility of experiments, where, for example, in one double blind test 70% of experimenters were found to smile at female subjects in a psychological experiment while only 12% smiled at males, thus influencing conclusions on sex role differences.

All these topics involve controversial science, in the sense that it is science at the time of formation, where conclusions are contestable and political and other prejudices can influence the interpretation of results. At this point science is inseparable from politics, and the Washington meeting went a long way to showing why and how this was so case by case. The sessions

chipped at the armour of the expert who set him or herself up as the final arbiter of truth, letting the politics go hang, and showed his and her relativity to social context wherever the science was ambiguous.

In recombinant DNA, for example, one commentator (Dr Susan Wright of the University of Michigan) concluded that "claims that the available evidence points in one direction or another seem premature. The view that the risks are minimal should be taken as the tentative opinion of a relatively small sector of the scientific community rather than a well established, theoretical position." Dr Philip Handler, President of the National Academy of Sciences, during a rather protectionist speech, spoke of "a crescendo of concern which is now diminishing somewhat. The public was frightened by tales of imaginary hazards reminiscent of the *Andromeda Strain* (a book written to be entertaining, not believed). And the statements of a handful of scientists, few of whom were close to this field of research, were taken as evidence that 'the scientific community is itself divided'".

In human sociobiology, Dr Edward O. Wilson, of Harvard University, believed he was articulating firm scientific evidence for the genetic basis of human behaviour, while one of his critics, Dr Stephen Gould, also of Harvard, argued that Wilson was doing no more than telling 'just-so' stories and not accumulating truth at all.

In the study of low level radiation effects as a cure for cancer among Hanford workers, Drs Thomas Mancuso, Alice Stewart, and George Kneale believed their results, though based on a quite small number of cases, to be of great human significance, while other scientists associated with earlier (and ten times smaller) estimates of radiative danger (principally from studies of the survivors of Hiroshima and Nagasaki) tended to dismiss the new data as alarmist.

Handler expressed the wish that "those engaged in such research would behave as scientists generally do and refrain from publication until they have completed a sufficient series of coherent studies to enable rational decision, rather than announcing each experiment in turn, generating public alarm that can neither be justified or assuaged". But such a view would seem to be idealist when the public want and need to know about possible dangers

as early as possible.

Handler quoted Maurice Arthus, one of the founders of immunology, who warned that when one had a dogmatic attachment to a theory "to persist in one's faith becomes in a sense a question of honour. Having adopted such a public position the protagonist . . . becomes like the attorney who defends a client in spite of the evidence of his crime. . . ." Handler added "how reminiscent of the recent history of DDT, diethylstilbestrol, cyclamates, and the SANGUINE antenna". Those of a different political persuasion might like to add others to Handler's list.

The most strident critic of the ivory tower was representative Richard L. Ottinger, member of the House Committee on Science and Technology, who spoke in a session on recombinant DNA. "DNA research has given me a fascinating insight into the arrogance and anti-democratic spirit of which the 'established' scientific community . . . has shown itself capable. Those who lobbied so hard to prevent the Congress from taking steps many of us are convinced need to be taken to protect public health and safety have shown that they think themselves omniscient and infallible. 'We are the experts' the saying goes, 'and you can't possibly understand whereof you speak'. I resent that, and I resent it extremely, and the American public will destroy you if you take that attitude".

Further, Ottinger claimed that scientists were not apolitical at all, but in fact rather vicious in dealing with their recalcitrant colleagues. Handler spoke of the "bitter irony that . . . the protest movement appears to be growing significantly from within science itself. These voices may comprise a very small fraction of the scientific community, but the malaise is real". This sounds dangerously like what Ottinger describes as "suggestions that have been made that scientists who themselves urge constraints may be alienated from the main stream. We have seen this happen, again, in the nuclear debate, and I sincerely hope that, this time, researchers into the problems and hazards of DNA will not find their research stifled". Ottinger added: "If I disagree on a policy matter with another member of the House, I'll tell him, and then I'll do my utmost to defeat his position. What I won't do is vilify or threaten him. Academic politics, on the other hand, is a reality too many attempt to avoid recognising."

Robert Walgate

AAAS

Race and IQ: Jensen retains fellowship

A MOVE to retract the fellowship awarded last year to the psychiatrist Dr Arthur R. Jensen was defeated at a council meeting of the American Association for the Advancement of Science last week. Jensen has long claimed that there is a heritable IQ difference between blacks and whites. His critics argue that his conclusions are based on a false interpretation of the data—and, in part, on false data—and accuse him of racialism.

The International Committee Against Racism (INCAR) petitioned the council on 16 February for 35 minutes, presenting six witnesses including three blacks: a disabled veteran, a Chicago construction worker, and a University of Denver neurophysiologist. The plea, in no uncertain terms, was that Jensen's arguments were non-scientific, in the tradition of white racialism, and unworthy of the AAAS. Jensen must be expelled, INCAR demanded.

INCAR, however, had no formal status at the meeting, and their motion was never put before the council. There were good reasons for this. Jensen was not present to defend his case. A decision to remove him would probably be unconstitutional under AAAS rules and the council was not as a body specifically competent in either psychology or genetics, opening it to the charge of acting on political and not scientific grounds. (Jensen's nomination had been presented last year by what should be a scientifically competent body, the psychology section of the AAAS).

The one black member of council present, Dr Bernard Gifford of the

Russell Sage Foundation, argued that for these reasons the council could not reject Jensen. But it could, he argued, reinvestigate his work, for since his nomination it had become clear that the twin studies of Sir Cyril Burt, on which a large part of Jensen's conclusions was based, were fraudulent.

Jensen's principal supporter at the meeting, Dr Lloyd Humphreys, a psychologist of the University of Illinois, attempted to outflank this move by claiming that Jensen's controversial work was only a small part of the evidence presented on his behalf to the psychology section last year. That caused a hiccup in proceedings until Gifford called for the citation which went with the Jensen nomination to be read out. It read: "For his research on testing individual differences, and intelligence: and particularly for features of his studies of intelligence testing". Gasps throughout the room.

Jensen likes to popularise his work. He wrote, for example, in the *New York Times Magazine* (31 August 1969): "There are intelligence genes, which are found in populations in different proportions, somewhat like the distribution of blood types. The number of intelligence genes seems to be lower, overall, in the black population than in the white". Yet according to Dr Stephen Gould of the Museum of Comparative Zoology, Harvard University, "Stephen Gould of the Museum of Comparative Zoology, Harvard, Jensen's conclusion is based on weak data and contains a fundamental fallacy."

Gould, a AAAS council member, told the meeting that "if you subtract



Disabled black veteran petitions AAAS council

the Burt data from Jensen's material you subtract the only controlled experiment . . . There is much debate on the rest". Further, he argued that while he accepts the existence of a 15-point IQ difference—one standard deviation—in the mean IQ of the black and white populations of America, this does not mean that the difference is genetic, even if the IQ is heritable within the black and white populations. "To assume so is a basic fallacy", said Gould. "I deal with this in the first lecture of my course on genetics".

The distinction is between 'within group' and 'without group' heritability. Height, for example, has a high heritability. The height of children is well correlated with the height of their parents. There is therefore a genetic factor at work. However, if one inbreeding group has a smaller height than another it does not mean they have "fewer height genes". It may well mean that the former group has a poorer diet than the latter. So with IQ: environmental factors cannot be eliminated even if within-group heritability is demonstrated.

In spite of such arguments, which were supported among others by Dr Mary Gonzales, the Vice-Chancellor of the University of Maryland, who has the problem 'on her doorstep', the only motion passed by the council was an anodyne prescription claiming that the AAAS has never adopted and never will adopt a racist or otherwise discriminatory stance. Dr Gifford, the black member, disputed the motion: AAAS involvement in the eugenics movement of the 1920s and 1930s made the motion a whitewash, he said.

The last chance for movement on the Jensen issue came with a motion to examine "the credibility of the scien-

No more fellows of the AAAS?

IN A move not entirely unrelated to the Jensen case, the AAAS council last week voted to impose a moratorium on all further fellowship elections, and to conduct a straw poll of the membership to determine if it wants to keep the institution. "It would be an act of grace and mercy to kill the fellowship scheme," said Dr Robert Allen, chairman of this year's Fellowship Committee. The AAAS has 128,000 members, (16,000 of them are fellows), but the process of electing new fellows is becoming a nightmare—and not just because of controversial elections.

Several of the sections of the AAAS fail to submit nominations at all, said Allen. This year seven of the

21 failed to do so, and only seven filled their quota. The evidence presented by the candidates on their behalf is meagre, and in the day the committee allots to selection there is time for only two minutes consideration of each candidate. No more time is spent as there is usually insufficient evidence to justify it.

The council will only use a poll of membership as a guide. If the members vote to abandon the fellowship, then a lengthy procedure has to be set up to change the constitution. If they vote, as expected, to keep it, the council will have to consider very carefully how to improve the election scheme to make it more equitable and less open to criticism.

tific work of Dr Arthur Jensen and its pertinence to the award of a fellowship to him". Gould called the motion "a somewhat abated formulation but probably the best we can do". Dr William Carey, Executive Officer of the AAAS, argued against it on the ground that if it were adopted he saw no end to the issue. That incensed one member, Dr James Silverberg, an anthropologist, who said "the reason we are spending so much time on this is that we left Denver [the location of the last annual meeting] with egg all over our faces". Silverberg condemned the earlier general motion as "a totally hypocritical statement. We have evidence that we knew what we were doing" and threatened to resign his own fellowship if the motion to reinvestigate Jensen did not pass. The motion failed by 25 votes to 10, a larger margin than the original vote last year to elect Jensen (28 votes to 24).

After the meeting, Gould expressed no surprise that the vote had gone the way it did. "The typical council member is an organisation man" said Gould. Gifford, on the other hand, was more disappointed. Humphreys had supported the investigation, retaining his belief in Jensen, and it had seemed to Gifford at that point as if the vote might go for the inquiry. Gifford is now believed to be planning to set up an *ad hoc* investigation of Jensen's work outside the auspices of the AAAS. He also believes the seeds of another Jensen affair may have already been sown with the election to fellowship this year of Dr Robert W. Fogel, author of the book, *The Economics of American Negro Slavery*. The book argues that the Negro slave was better off and happier than is usually thought. "Fogel is a friend of mine" said Gifford "but this book doesn't represent his best work".

Robert Walgate

Special treatment for Cuba and Mongolia

CUBA and Mongolia are to receive preferential treatment as 'less-developed' countries, under the Comecon plans for scientific and technical cooperation. This was announced by the Cuban Deputy Premier, Belarmino Castella Mas, in his opening speech at the eighteenth session of the Committee for Scientific and Technical Cooperation; the first meeting of this committee to be held in Cuba.

Comecon cooperation in science and technology, as in all other branches of the economy, is officially governed by the 'Complex Programme of Further Intensification and Perfection of the Cooperation and the Development of Socialist Economic Integration among the Comecon countries' of 1971.

This laid down long-term objectives (up to 1990), including the formation of international scientific and technical associations, joint laboratories, coordination centres, scientific and technical councils and study groups.

The programme, however, seems to have proved too grandiose in practice so the January 1976 meeting of the Committee on Scientific and Technical Cooperation stated that for the present, scientific cooperation will be largely devoted to research projects "which cannot be solved by individual countries".

The recent session of the Committee had as the main item on its agenda the accelerated development of science and technology in Cuba. Comecon planning policy tends to follow current Soviet doctrine; hence the current emphasis is on practical applications rather than basic research. Development programmes will include the increased use of modern technology in the cultivation and processing of sugar cane, 'agro-industrial' development of citrus fruit production, the use of nuclear energy (a power station is to be built in Cuba by the Soviet Union), solar energy, and the development of the electrical power system. Plans to increase Cuban nickel and cobalt production to 130,000 tonnes per year (almost a quarter of the total world nickel output) have already been under way for a year.

Details of the financing of these projects are not available. Rather remarkably there is no central Come-

con fund for financing joint research projects, and all cooperation projects have to be based on *ad hoc* financial agreements between the member countries concerned. It is noteworthy, however, that while Eastern Europe has a considerable beet sugar industry, there seems to be an upper limit to further expansion—increased use of fertiliser providing larger gross tonnage of beets but no larger yield of sugar.

Investment in the Cuban sugar industry, whether or not repaid directly in kind, is likely to benefit the flagging food industry of Eastern Europe. And the development of Cuban nickel will provide a much-needed component of high-quality steels and high-strength nonferrous alloys.

To date, Cuba's contribution to Comecon has consisted largely in hosting meetings of the Executive Council and various committees, provision of a site for a satellite-tracking base, and the export of certain commodities (some of which, like cigars, are considered by the senior partners as "inferior"). Likewise, Mongolia's main contribution has been in geological surveys which will ultimately provide raw materials for the bloc.

The new "preferential treatment" of Cuba and Mongolia while undoubtedly beneficial to the development of their economies, still seems ultimately directed at the over-all good of Comecon *in toto*. The plans for the 'less developed' members clearly reflect the ultimate aim of an integrated economy.

Vera Rich

Denmark follows UK on DNA guidelines

DENMARK is preparing guidelines for research involving genetic engineering. At the end of last year, the Danish Parliament offered broad approval to the recommendations laid out in a report of the 'Registration Committee for Genetic Engineering' and assigned to the Committee the task of working out preliminary guidelines for experiments involving recombinant DNA.

The Committee was set up by the Danish Research Councils in July 1976 on lines similar to those of committees in other countries. Its task was to formulate proposals for the Commission for Genetic Engineering, a permanent organisation to control genetic engineering in Denmark, and then to function as the Commission pending its establishment. The Committee is made up of representatives from the National Health Service, the Agency for Environmental Protection, the pharmaceutical industry and the Ministry of the Interior.

To find out where genetic engineer-

ing research is done in Denmark, the Committee inquired into 195 public and private laboratories and organisations and found that no such research has yet been done. Two laboratories, however (one of them the Carlsberg Laboratory), intend to do experiments involving genetic engineering very soon.

Many of the report's recommendations are in line with those of the UK Williams report rather than the US NIH guidelines. This suggests that the Committee has taken into account the wish of the European Science Foundation (ESF) and the European Molecular Biology Organisation (EMBO), that regulations should be equivalent, if possible, in all European countries.

As Denmark has only very limited resources for building high containment laboratories, Danish scientists will probably carry out high-risk experiments in common European laboratories (for example, the EMBO laboratory under construction in Heidelberg). It is therefore important that they

should be subject to the same regulations as those operating in such international laboratories. The Committee also feels that if Denmark sticks to common guidelines, it will be able to call on experts in the ESF's 'Liaison committee on recombinant DNA research'—on which it is represented—for advice with specific genetic engineering problems.

The report's recommendations aim at controlling public as well as private research and ensuring that the community has the power to regulate the use of any results from genetic engineering research which may pose hazards to the environment, animals or man. And that power, the report says, should eventually be vested in the Commission for Genetic Engineering when it is set up.

All research and production using genetic engineering will then have to be made known to the Commission and particularly hazardous procedures will not be allowed to go ahead without the Commission's approval. Laboratories will have to be approved by the Directorate of Labour Inspection, or by a public or private institution authorised by the Directorate in collaboration with the Commission. People working with genetic engineering will have to be adequately trained; accurate records will have to be kept of all experiments; accidents will have to be immediately notified to the Commission and laboratories will have to be inspected regularly.

A point which the Registration Committee suggests should be looked into further is the question of whether existing Danish law (the Epidemics Act 1915, the Working Environment Act 1975, the Environment Protection Act 1973) affords a sufficient supervisory authority to inspect laboratories or whether special legislation will be needed. Eventually, however, recommendations from the European Economic Community (EEC) will have to be taken into account on the latter point. As yet no decision has been taken on which central authority the Commission should be placed under, but the Registration Committee suggests that the National Health Service, which supervises other forms of microbiological research, might be the most suitable.

The Registration Committee's next task will be to collaborate with the National Health Service, the Directorate of Labour Inspection and the Agency of Environmental Protection to work out preliminary guidelines for registration, supervision, approval of laboratory facilities and so on. In the meantime it will keep the government and administrative authorities informed of developments abroad.

Sven Godtfredsen

First Ratan results published

THE first results of experiments with Ratan-600, the 600 m radio-telescope of the Soviet Academy of Sciences, have recently been published. The experiments are essentially exploratory and performance trials ranging from solar to extra-galactic observations.

The Ratan-600 represents a new generation of radio-telescopes. Its design parameters are impressive: information capacity 3×10^{12} bits, limiting resolution 2 arc sec, accuracy of definition of coordinates 0.01 sec², limiting flux density 0.01 Jansky, limiting brightness temperature 0.03 K, sky coverage 70–80% with scanning time 100 days. Ultimately it is hoped that Ratan-600 will not only provide direct observational results, but will serve as a basis for experiments estimating the intensity of gravitational waves and confirming general relativity on the basis of the bending of rays in the gravitational field of the sun. Although these sophisticated experiments lie in the future, a number of interesting results have already been obtained.

The high resolution and large collecting area of RATAN-600 permit the observation of solar "pores"—areas of limitingly weak activity which will later develop into sunspots. It also allows the radiogranules, first discovered during the transit of Mercury in 1970 to be monitored in non-eclipse conditions and for several frequencies simultaneously. A programme of joint radio and optical investigation of solar

granules has been initiated and preliminary investigations obtained for their magnetic fields.

Likewise, the high resolution made it possible for RATAN-600 to receive radio-emissions from all four of the "Galilean" satellites of Jupiter, in spite of the intense radio-emission from the planet itself. These observations—in December 1976 and January 1977, gave the first results ever for Io, the closest, and Europa, the nearest, of the four. For Ganymede, Callisto and Europa, the results were consistent with thermal emission only, showing no signs of the intense radiation bands observed for Jupiter itself. For Io, however, the result was "totally unexpected"—radio-emission is several times greater than the expected thermal value. It is claimed that this implies that Io possesses a magnetic field and intense radiation bands.

RATAN-600 is also participating in two joint Soviet-Australian projects, on quasars and on early-type emission line stars. Signals have been received from quasar OQ 172, the most remote object in the universe (red shift 3.54). Its present programme also includes observations of the galactic centre, multi-frequency mapping of radiogalaxies, and the first stages of a sky survey in the centimetre and millimetre ranges, which will take 10 years to complete and should provide a radio equivalent of the Palomar sky survey.

Vera Rich

Bangladesh plans nuclear research centre . . .

BANGLADESH Atomic Energy Commission, in its efforts to develop peaceful applications of nuclear physics and technology, has drawn up an extensive plan to establish a nuclear research complex in Bangladesh. It will comprise seventeen nuclear and allied projects. A 259-acre site twenty-five miles north of Dacca is already under development. The laboratories are to be integrated to share facilities and also to create an environment conducive to the formation of interdisciplinary teams among the scientists of the Commission.

Mr H. F. S. Bittencourt, Deputy Director General of the International Atomic Energy Agency, while visiting Bangladesh in connection with the inaugural ceremony of the Institutes of Nuclear Technology, Nuclear Medicine, Electronics, Irradiation and Pest Control, the National Com-

puter Centre, the Institute of Space and Atmospheric Research, and other institutes, expressed his satisfaction over the plans and assured all possible help in matters of providing the Commission with a small nuclear reactor. It is expected that the first phase of the work will be completed by 1982, but Bangladesh still needs a 10 to 20 MW reactor, which the BAEC hopes to get as a donation from some friendly country. Bangladesh, however, is required to obtain a nuclear safeguard clearance before obtaining a nuclear reactor and it is hoped that the IAEA will extend the necessary help.

Two irradiators each of 50,000 Curies for food irradiation and medical projects are to be procured from Canada. The first one which is expected to arrive in June next, will cost about US\$6,000.

M. Kabir

Soviet schools tied closer to technology

THE relationship between science and the needs of the national economy is a matter of constant theoretical discussion among the Soviet planners. So too is the relationship between education and the demands of expanding technological growth. The latest decree of the Central Committee of the CPSU and the Supreme Soviet on the further improvement of secondary education reflects a new concern with these problems.

The current doctrine stresses that science must serve the needs of the national economy—with Brezhnev's famous caveat that "there is nothing more practical than a good theory!". Even interplanetary probes are announced to have been launched "in accordance with the needs of the national economy". During the last few years, several scientific bodies—for example, the Far Eastern Science Centre—have been publicly censured for failing to orientate their research to the immediate needs of production. The closer coordination of research and the needs of industry is a matter of pressing concern, and has led to the establishment of various problem solving institutes closely linked to production enterprises in their areas.

Not surprisingly, therefore, the new decree on education extends the same process to the secondary school level. Referring to the "Leninist" principle of a "single, labouring, polytechnic school", the decree notes that, at present, school-leavers' skills and outlook do not correspond "to the requirements of social production and scientific and technical progress". The decree continues "Many school leavers go out into the world without the necessary preparation for work, without sufficient information about the basic professions of the masses, and they experience difficulties in the transition to working for the national economy".

This stress on preparation for work suggests a return to the Khrushchev era, when parties of school-children, carrying out what was officially described as "labour training" in factory and collective farm, succeeded only in antagonising and disrupting the workers, who grumbled that they were expected to act as unpaid nursemaids, in addition to their official duties. In fact, however, little is being done on these lines. After the fall of Khrushchev, labour training was quietly phased out except in senior classes; the present decree urges only that pupils of the ninth and tenth classes (16 to 18 years old) should have their "labour training" increased from two to four hours per week (within the limits of the timetable) and that this training should correspond to the facilities and

needs of local production. Special emphasis is placed on vocational training, especially for those leaving after the eighth class (Soviet compulsory education was officially extended to ten classes some years ago, and it is interesting that the option of leaving after eight years has still not been completely



Vanya has stolen the exam questions! I'm not sure whether to fail him or give him an 'A'!

phased out).

Shortcomings in syllabuses and textbooks are stressed; in a number of cases, it is said, these are overloaded with "superfluous information and matters of secondary importance". The proposed changes will ensure that courses include the necessary amounts of the rudiments of the sciences with information on the relationship of the subjects studied to the needs of the economy. At the same time, the total work-load of pupils is to be decreased to 24 hours in the first to third classes, rising gradually to 32 hours in the tenth class. Excessive homework is to be eliminated.

Vocational guidance will become the special responsibility of an Interdepartmental Methodological Council of the Ministry of Education, with a "base enterprise (organisation)" attached to every school.

An increase in the direction of young university graduates into school teaching is envisaged; in compensation, however, the title of "People's Teacher of the USSR" has been established for those who distinguish themselves in the field of education.

Finally, and perhaps most important, school children will at last receive free textbooks loaned to them by the school, and special funds are to be allotted to enable the schools to buy the necessary book stocks. □

Japan makes new study of its science

JAPAN'S total R&D expenditures rank as fourth largest in the world, according to the latest figures released by the Japanese government in a white paper on science and technology, but net growth has ceased, once inflation is taken into account. Total expenditures for fiscal 1975 (the last year for which most figures are given) were 2,622 billion yen, roughly equal to \$8.8 billion or £4.0 billion at that time. This figure was about a quarter the expenditures of the United States and one-third those of the Soviet Union for R&D that year, placing Japan just behind West Germany and just ahead of France. Net expenditures were 2.7% higher in 1975 than 1970, but they peaked in 1973.

Expressed as a proportion of the national economy, Japan's R&D expenditures were 2.06% of the gross national income, compared to 4.8% for the Soviet Union and 2.55% for the United States. The most important comparison to other countries, however, involves identifying the source of these funds. Government expenditures account for only about a quarter of the total in Japan, compared to nearly half the total in most other developed countries. A much larger proportion of

research is thus funded by private industry, which profoundly affects the nature of projects undertaken. Corporations, for example, spend more than three times as much on R&D as either universities or research institutes.

As one would expect, this private funding produces a heavy bias in favour of what would be called applied research and development, in the West. However, this tendency is not readily apparent when one first examines the figures of the white paper—theoretically Japan spends 14.2% of its science and technology funds for basic research, compared to 12.9% in the United States and 8.0% in the United Kingdom. A better understanding of this paradox emerges after examination of other government documents to find what sorts of projects are included under the definition of "basic" research. Many of the areas involved, such as materials development, do indeed require investigation for longer periods than one might ordinarily consider reasonable for "applied" research, but a specific commercial application is usually envisaged. Even by their own definition, however, the Japanese are rapidly decreasing the proportion of

funding devoted to basic research.

The distribution of manpower among various sectors parallels the division of funds, with corporations employing substantially more researchers and technicians than both universities and research institutes combined. For the calendar year 1976, total Japanese R&D manpower was 488,000, divided into 53.3% researchers, 16.2% assistant researchers, and 18.3% technicians. If one allows for differences in defining "researcher", the number of scientists and engineers is about half that in the United States and three times that in the United Kingdom.

Since much of the technological underpinning of Japan's most successful industries has been borrowed and adapted from abroad, a recurring theme of recent science policy statements has been the need to improve domestic research so that more in-

novations could be produced in Japan. One indication of progress has been a strong positive shift in Japan's 'balance of patents', with more Japanese applying for patents abroad than foreigners applying for patents in Japan. But a more important figure is the balance of cost between buying and selling rights to technology, and here Japan runs a large and increasing deficit. Using after-tax figures for all enterprises involved in such trade, 1976 receipts were only 20.4% of sales, down from 22.6% the year before. Clearly Japan remains strongly dependent on research conducted in other countries for much of its industrial technology.

Finally, when comparing Japanese research to that of other countries, the question of professional salaries is often brought up, only to degenerate into a welter of contradictory statements. Interviews with the officials of the

Science and Technology Agency who produced the white paper provide the following basis for limited comparison. In 1976 a 40-year-old researcher had an average monthly income of 225,538 yen, then equivalent to about \$760, including some overtime. A base annual salary would thus come to \$9,125, but to this must be added a lump-sum "bonus" of nearly six months' wages, bringing the total to \$13,687. Since Japanese income taxes are quite low for this salary range, the researcher's after-tax income would be equivalent to that of an American or European with substantially higher gross wages. But since pensions in Japan are substantially less than those abroad, the researchers would have to set aside more of his income for retirement, which generally comes at a younger age than for his foreign colleagues.

John Douglas

THE dispute about gene-splicing typifies some of the current hostility towards science. There is one important exception: gene-splicing dangers are purely theoretical. Recombinant DNA has never been accused of producing a tumour in the bladder of a rat, or of making a brown pelican lay a soft-shelled egg. Nevertheless, the rank-and-file defenders of the environment respond to the summons of their leaders, and sally forth to do battle with the mysterious shadows of manipulated molecules that could envelop and ruin our 'space-ship Earth'. Three billion years of organic evolution will be like an evening we are told, when the fantastic new life-forms escape from the test-tubes.

Perhaps so. In any case, I have been fascinated by the eagerness with which lawyers, clergy, and lawmakers enter the dispute. It was to be expected that the issue would be seized by bureaucracies for purpose of self-proliferation. There were good precedents. The return of predictably lifeless rocks from the surface of the moon gave rise to a scramble for authority as to who should shield us from the dangers of contamination. The officials in charge of plant quarantine demanded that the samples be tested for wheat-rust spores.

The more successful assaults on technology are based on projected rather than actual hazards. The real catastrophes don't seem to be as interesting. Dams collapse and many people are drowned. These tragedies are soon forgotten, and dams for hydroelectric power continue to be built. Plutonium is a better target; it is new, it is dreadful, and it must be

exorcised—just in case. Cigarettes kill thousands with lung cancer and emphysema, but advertisements in the United States still assail us with

Wandering minstrels



THOMAS H. JUKES

gigantic pictures of nicotine-loving cowboys and liberated women smokers. Currently, the carcinogen of interest at the US Department of Agriculture is bacon, not subsidised tobacco.

The most unusual of all targets is the phantom menace of genetically-engineered organisms; a legislated, institutionalised and regulated figment of the imagination. People who know least about DNA are the most eloquent among those who fear it. They don't quote Jim Watson, who, on the subject of recombinant

DNA, was reported to have said recently: "Science is good for society. We are being attacked by everyone who doesn't have the guts to go ahead. The dangers of this thing are so slight—you might as well worry about being licked by a dog".

He was too late. The molecular biologists did themselves in at Asilomar. After this, it was a foregone conclusion that DNA would be placed under official guidelines. Like a layer of molasses, the non-ebbing tide of regulations has crept over the field of gene-splicing. How can a danger ever become trivial if the livelihood of an echelon of bureaucrats depends on writing rules to cope with it? The officials now need to demonstrate their public zeal.

In *The Mikado*, the Emperor of Japan is angry because there haven't been any executions lately in the town of Titipu. A victim must be found, or, by imperial decree, Titipu will be reduced to the level of a hamlet. So the civic leaders schedule a simulated beheading for a wandering minstrel, Nanki-Poo.

Recently, in Boston, a victim was apparently needed to show that the National Institutes of Health guidelines on gene-splicing were being enforced. The prototypic Nanki-Poo was Charlie Thomas, who had been caught red-handed for filing a document too late. Watson was unkind enough to liken such infractions, when committed knowingly, to "cheating at tiddlywinks", but this particular sin seems to have been inadvertent. Anyway, Boston was saved, and let us hope that, like Nanki-Poo, Thomas will escape from the Lord High Executioner.

correspondence

NERC support for UK geophysics not adequate

SIR,—“Geophysics is a sub-division of geology”. This comment was made by a representative of the UK Natural Environment Research Council (NERC) at the November Royal Astronomical Society meeting on how NERC supports geophysics in the UK. The comment probably revealed more about NERC's attitude to geophysics than all the numbers rained upon us.

£6 million, or 14% of NERC's annual budget, was spent, we were told, on geophysics. In the three relevant national institutes, the support for geophysics as a percentage of total expenditure is 19% at the Institute of Geological Sciences (IGS), 15% at the Institute of Oceanographic Sciences (IOS) and 35% at the British Antarctic Survey (BAS). Ten per cent of the £6 million was spent on geophysics in universities. Further, NERC supports eight research ships on which geophysics takes approximately 13% of the cost.

So an apparently convincing case can be made that support for geophysics is adequate. Most of the geophysics done in the NERC institutes and ships, however, is survey geophysics. In IGS and BAS it is a powerful tool to help the geologist—this is where the idea that geophysics is part of geology comes from (*Nature* 249, 794, 1974). In IOS, solid earth geophysics is the mapping of the ocean floor. But geophysics is a much bigger subject than surveying the external layers of the earth. Geophysics is about ideas on the nature, origin, and evolution of the Earth and planets, and in this field we have shown that we can be international leaders—but for how long with NERC's present policies?

Take one particular subdiscipline, palaeomagnetism. British scientists have led the way in creating this field which has brought about a revolution in Earth science. At present small groups at five universities are doing innovative work, fully competitive with work abroad, but they are starved of research students, and recently the Research Grants Committee decided that the all important instrument for the next decade's work, the cryogenic magnetometer (£20,000), is to be shared between these centres. In comparison, vast sums of money are awarded whenever NERC's commitment to BAS or research vessels needs justification by university research activities. One may legitimately doubt if such projects would be funded to the extent they are on their fundamental scientific merits, but commitments are made, in the case of the BAS, for political reasons.

Nor is the problem simply at the research grant level. Instead of allotting a number of studentships to departments to distribute to those who show exceptional promise, all members of geo-

science departments are now invited by NERC to put forward projects (described in three or four lines on the appropriate form). In awarding studentships, NERC selects projects thought most suitable for training, although the numerical distribution of studentships among departments follows an unchanging pattern. Thus the training awards committee for the geological sciences tells heads of departments which projects to put their research students on, without, of course, intimate knowledge of the department's research programme or of the prospective supervisors. Quite apart from this objectionable inroad into the independence of university departments to decide what courses are best for their students, the system continually throws up insoluble problems; a student wants to work with A, whose project this year has been turned down; nobody wants to work with B whose project has been approved.

The scheme is wisely not followed by SRC, but it doubtless gives NERC a chance to say in Whitehall that the universities are being guided into more 'relevant' research; indeed Dr Twinn of the council said at the meeting that university support is a 50-50 split between applied and pure. This shows a fundamental misunderstanding of the purpose of university research training; it is the intellectual challenge in a field which is relevant to the suitability of training.

Of the studentships awarded by the Committee, 14% are allotted to geophysics. This is not the right balance between a subject in which exciting developments have occurred and the more traditional field of geology. The Department of Geodesy and Geophysics at Cambridge has for as many years as I can remember been allotted two studentships yearly; so has my own department at Newcastle, which the university decided years ago to build up as a large centre of geophysical research. Many less internationally known geology departments receive three or four studentships and Imperial College Geology Department regularly gets over 12. Thus members of the staff of my department have a very much poorer chance of supervising a research student than they would in these more traditionally minded departments.

But as geophysicists have been only a tiny minority on the research grants committee and only one pure geophysicist sits on the training awards committee (no geophysicist has been chairman of either) and as this pattern and the policy are self-perpetuating and NERC headquarters seems entirely satisfied, no change is likely without open debate. In the National Science Foundation's solid earth division the support for geophysics, geochemistry and “traditional geology” is in the ratio of 2:2:1 respectively.

Yours faithfully,
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Tidal power schemes in Korea

SIR,—While we debate tidal energy schemes in Britain, the Korean government intends to have a tidal power station in operation in Korea by 1986. The group responsible for this is the Marine Science division of the Korean Research Institute for Ship and Ocean, previously known as KORDI (*Nature*, 254, 551).

The western coast of Korea is heavily indented with many large inlets. There are numerous off-shore islands and a tidal range of up to 10m. The country is mountainous with limited fossil-fuel resources and with most of its hydro-electric energy developed. It is heavily dependent on imported oil. A nuclear programme is also being implemented.

For tidal energy production there are several promising sites under examination and we recently visited one of these in Chung Nam province. During this visit we observed the construction of the Sap Kyo tidal barrier Asan-Gun. This is the third sea-arm closure to be made in this area since 1972 for land reclamation and water-resources schemes.

The Sap Kyo barrier is to be 3.4km long, built in water depths of up to 18m, though the greater part of its length is in 8-12m depths. The barrier is being constructed from rock, sand and earth-fill placed over a polyester and hessian bottom protection layer which in turn overlies a mean depth of 12m of silt and sand. The maximum tidal range at the site exceeds 10m and the impounded basin area exceeds 20km².

The closure of the sea-arm is being undertaken by Korean contractors, without special construction methods such as cableways or float-in caissons, although the closure velocities will rise to 8 ms⁻¹. The closure will be made next April, only 17 months after the start of the construction. Time for completion of the whole project, which includes a 12m wide roadway at 8.5m above mean sea level and a sluicing structure 137m long with six shell roller gates, is three years, at a cost of less than US \$30million.

The cost of this embankment is about US \$9,000 per m built at a rate of 3.1 m per day overall*. These are, of course, only approximate measures of performance, but they may be compared with recent estimates made for the Severn estuary closure-barrier of about US \$300,000 per m built at a rate of 2.1 m per day overall. The two schemes are not, of course, strictly comparable as the Severn closure-barrier has many concrete caissons incorporated in it. Nevertheless, if the Lavernock Point-Flat Holm section alone is considered, where the projects are roughly similar, the ratio of costs is still almost 6:1.

Yours faithfully,
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*All data from Korean government publications.

news and views

Embryonic and regenerating patterns

from Jonathan Cooke

If the productivity of a scientific model may be judged from the energy with which workers subsequently attempt to refute it experimentally, the recent 'clockface' model of French, Bryant and Bryant for the control of pattern in a variety of developing animal structures (*Science* 193, 969; 1976) must rate as highly successful. Equally characteristic, for a highly charismatic model, is the way in which its proponents refuse to accept at face value the counter-evidence offered by others, and attempt instead to preserve the original concept intact in essence, by tinkering with it in relatively superficial ways. This is as it should be, as it provokes (not to say irritates) the community into yet more experimental work.

The model was proposed as a way of understanding the formation of pattern elements during the regeneration of a range of animal structures including already formed insect and then vertebrate (newt) limbs. There seemed to be a single set of rules governing the kinds of structure produced after simple amputation or various cutting and splicing operations, whether on the leg of a cockroach, the forelimb of an amphibian, or the imaginal disc of a *Drosophila* larva (see also Bryant & Iten, *Develop. Biol.* 50, 212; 1976).

Briefly, the proximo-distal and circumferential dimensions of the limb pattern are believed to be specified by a system of radial coordinates on which the cells are assigned positional values determining which differentiated structure they will form.

Each small region has a circumferential value (numbered arbitrarily one to twelve, hence 'clockface') and a radial, or proximo-distal value. In insect appendages, the surface concerned is literally the cylindrical sur-

face of epidermis whose cells lay down the pattern elements (bristles, cuticle types, and so on) that can be recognised, while in vertebrate limbs it seems to be the cylinder of mesenchyme (and not the skeletal core or the dermal surface) that embodies the position values. For both limb systems the radial co-ordinates run from base to tip of the appendage (proximal to distal), converging at the latter.

Two rules are postulated for the generation of new pattern parts after the removal of tissue or the artificial bringing together of tissue with different positional values. The first is that of intercalation. When tissues of different co-ordinate values are brought together, growth occurs at the junction to lay down tissue of all the intervening values in series, as defined by the shortest route around the arbitrarily numbered clockface and by any missing radial (proximo-distal) levels. But the second rule is that if the radial series is left incomplete distally (rather than simply having a gap in it wherein intercalary growth can occur), the regenerative growth to re-complete the series will only occur if cells including the entire circumferential (clockface) set of values can be present at the healed wound.

It is this second complete circle rule which has been the recent subject of experimental attack, but it is worth asking first why such a hypothesis has so excited and/or irritated those re-searching into the unknown mechanisms of pattern formation. It challenges the idea (which was beginning to gain the status of 'knowledge') that cell position is always coded by some kind of gradient. The shortest-route intercalation rule, applying as it does anywhere around the clockface seems to negate the gradient idea since a 'cliff' or singularity, with special behaviour in grafting operations, would be required at the junction of a graded scalar series of values within the cylindrical tissue.

Bryant and Iten, in the paper cited

above, use the complete circle rule to account, in an undeniably impressive way, for the asymmetry, position and orientation of supernumerary limbs that grow near the graft-host junction when well formed regeneration blastemas are transposed from previously amputated limbs to other, host limb stumps in newts, so as to cause graft-host discordance with respect to anteroposterior axis only, dorsoventral axis only, or both axes (by 180° rotation of blastema on its own stump). Detailed rationale and diagrammatic explanation of the results, according to the model, can be forced in Bryant and Iten 1976, or in Bryant (*Symp. Soc. Devel. Biol.* 3 (ed. Ede, Hinchcliffe & Balls), Cambridge University Press, 1977). Briefly, each supernumerary is seen as a new onset of growth, producing all distal tissues, provoked where adjacent intercalations in opposite directions round the 0-12 series generate new, additional clockface sets of cells at graft-host junction. The finding that there are only two sites for supernumeraries, instead of the formally expected four after a 180° blastema rotation, is accounted for by postulating uneven distribution of the 0-12 series of values in real tissue space round the limb. Why not? Robust models should easily digest such small doses of *ad hoc*ery, as this one does.

Digestion fails however (at least, mine does), at the recent discrepant results reported by Bryant (*Nature* 261, 44; 1976) and now Slack and Savage (*Nature*, 271, 760; 1978), following what seem to be similar experimental tests of the model. Just how similar are the two experiments? Bryant prepared, surgically, compound differentiated limbs composed of two posterior halves and possessing, on the model, two similar partial sets of clockface values healed in mirror image symmetry. The observed failure of regeneration, after amputation of such limbs, was as predicted from the model: no possibility of generating

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complete clockfaces, so no new distal tissue production. But the double posterior limbs discussed by Slack and Savage were produced not by surgery in the adult, but by operations in the embryo that altered the antero-posterior polarity of the limb-forming tissues. It is known (see Slack, *J. Embryol. exp. Morph.* **39**, 151; 1977) that flank tissue specifies the polarity of the developing forelimb, and by transplanting a strip of flank to the anterior margin of the limb-forming tissue they can produce symmetrical double-posterior limbs. Such limbs

would have to possess, on the model, partial, mirror-image duplicated sets of positional values; yet on amputation they frequently regenerate a double-posterior pattern similar to that of their initial development. This is in clear contravention of the complete circle rule.

French *et al.* do suggest, in the initial *Science* paper, how the apparent evidence that organ rudiments are successively polarised in two Cartesian-type axes during early development (see the above Slack articles and for example Harrison, *J. exp. Zool.* **32**, 1;

1921), may be reconciled with later regeneration behaviour of limbs initially developing mirror image symmetry in embryonic life, and indeed the possibility of stable initial development of such limbs at all, remains a severe challenge to the clockface model. Perhaps we should not expect to understand, in one breath as it were, the initial genesis of patterns in growing fields in embryos and then their later capacities in regeneration (if they show such capacities, which of course the limbs of higher vertebrates do not). □

Ubiquitous neuronal peptides

from John Hughes and Leslie Iversen

The 1978 Winterschool of the European Training Programme in Brain and Behaviour Research was held on January 8-14, at Zuoz, Switzerland on the topic 'Neuronal Peptides'. The Chairman was J. J. Dreifuss, Geneva.

DURING the symposium, participants attempted to come to terms with the avalanche of new data that threatens to overwhelm those involved in this field. At the latest count, at least 20 peptides are known in mammalian brain with distributions and properties compatible with neurotransmitter functions.

The widespread and often unexpected distribution of neuronal peptides has been demonstrated recently in several laboratories. F. Vandesande (University of Ghent) illustrated the powerful resolution available with immunohistochemical staining techniques. By using carefully purified antisera it is possible to stain selectively hypothalamic neurones containing the closely-related pituitary peptides oxytocin and vasopressin. Furthermore, some vasopressin neurones seem to exist within extrahypothalamic areas of brain. Similarly, B. Flerko (Pécs) described the existence of the hypothalamic releasing hormone LHRH in neurones in various extrahypothalamic areas, and M. Palkovits (Budapest) provided radioimmunoassay results from microdissected brain regions supporting the existence of this and other hypothalamic releasing hormones outside the hypothalamus. Unpublished results of Lundberg and Hökfelt (University of Stockholm) indicate the presence of nerve fibres in the cervical vagus containing substance P, en-

kephalins, gastrin, somatostatin and vasoactive intestinal peptide (VIP). The nodose ganglion contains VIP and somatostatin-containing cell bodies and these together with substance P cells presumably form part of an afferent system, whereas enkephalin and gastrin seem to be contained in efferent fibres originating from cells within the brain. Equally intriguing is the possible coexistence of somatostatin and noradrenaline in some sympathetic ganglion cells (Hökfelt *et al. Proc. natn. Acad. Sci. U.S.A.*, **74**, 3587; 1977). The coexistence of more than one putative transmitter within a neurone clearly has important implications for generally accepted concepts regarding the release and action of neurotransmitters.

J. Hughes (Imperial College, London) described studies on the biosynthesis of the enkephalins, suggesting the need for ribosomal synthesis and the probable existence of a larger polypeptide precursor. There is evidence that a single large polypeptide may represent the common precursor for ACTH and β -lipotropin in the pituitary, and the latter peptide can in turn give rise to α -MSH and β -endorphin. There are other examples of families of peptides originating from a common precursor. Thus, radioimmunoassay results suggest the presence of both cholecystokinin (CCK) and its carboxy terminal octapeptide (CCK-8) in the brain (Dockray *Nature* **262**, 92; 1976; Muller *et al., Proc. natn. Acad. Sci. U.S.A.*, **74**, 3035; 1977). The cerebral cortex is particularly rich in these peptides, which is of interest since this area is relatively sparsely innervated by other known peptidergic systems. Gastrin and CCK probably share a common evolutionary precursor (Larsson & Rehfeld *Nature*, **269**, 335; 1977) and it is not surprising that gastrin-like immunoreactivity has also been reported in brain (Vanderhaegen *et al. Nature*, **257**, 604; 1975).

Turning to the possible functions of CNS peptides, J. S. Kelly and L. L. Iversen (MRC Neuropharmacology Unit, Cambridge) discussed the possible involvement of substance P as a primary sensory transmitter involved in the transmission of pain, and the interaction of enkephalin-containing neurones with pain-transmitting mechanisms at the spinal cord and brain stem level as a possible 'gating' mechanism (P. Wall, University College, London). R. F. Schmidt (Kiel) described the various chemical mechanisms involved in activating and sensitising pain fibres in the periphery. A special category of muscle afferents responds to painful stimuli and to pain-inducing chemicals such as 5-hydroxytryptamine or the peptide bradykinin. The action of bradykinin may involve a prostaglandin intermediate, since its effects on pain fibres are blocked by aspirin.

J. Fitzsimmons (University of Cambridge) reviewed the remarkable CNS effects of angiotensin II in eliciting drinking behaviour in most vertebrates. An outstanding question here is whether such effects are normally elicited by the circulating peptide acting on targets in the brain lying outside the blood-brain barrier, or whether an intrinsic CNS renin/angiotensin system is involved. T. B. van Wimersma Griedanus (University of Utrecht) described results obtained with D. de Wied showing that ACTH fragments and particularly vasopressin are able to delay the extinction of learned behaviour in animals. Whether the central actions of vasopressin are related to an intrinsic system of neurones containing this peptide in brain, or whether the pituitary vasopressin system is involved is again unclear.

The Winterschool showed that the field of neuronal peptides remains a rapid growth area; many unanswered questions have been raised by the sudden discovery of this large group of new putative chemical messengers in the brain, and there will certainly be more surprises to come. □

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Methanogenic bacteria: a new primary kingdom?

from J. F. Wilkinson

BIOLOGISTS until recently have divided living organisms into two main groups—plants (including bacteria) and animals. The development of the electron microscope made it clear that a more fundamental dichotomy existed in terms of cell structure and the prokaryotic and eukaryotic types of organism were defined. This latter division has been amply confirmed at different levels of cell organisation, but it has usually been considered quite wrongly to represent a fundamental division into two primary phylogenetic groupings—the Prokaryotae and the Eukaryotae. However, it has become clear in recent years that a eukaryotic cell has a composite nature (for example, Stanier *Symp. Soc. gen. Microbiol.* **20**, 1; 1970). It is generally assumed that chloroplasts and mitochondria are descended from a quite different ancestral line from the rest of the eukaryotic cell, which therefore represents the eventual result of an essentially symbiotic relationship between two cell types. Thus it becomes necessary to assume that there may have been at least two ancestral cell types represented by the prokaryote and the cell type that led to the eukaryote; mitochondria and chloroplasts may or may not represent two further cell types.

Can we obtain any information concerning the evolutionary relationships between such ancestral cell types and is it true that all present-day prokaryotes and eukaryotes fall into two basic groupings? To answer these questions, it is necessary to find suitable structures which are common to all living organisms and to compare them. The more conserved such structures are during evolution, the more suitable they are for the purpose. The molecular biologist naturally seeks some defined polymer in which the base sequence can be determined. A good case can be made that the most satisfactory single molecule available for this purpose is rRNA; it is a component of all self-replicating systems including chloroplasts and mitochondria, it is readily isolated and its sequence seems to change only slowly with time allowing the detection of relatedness amongst very distant species (Sogin *et al. J. molec. Evol.* **1**, 173; 1972; Woese *et al. Nature* **254**, 83; 1975; Fox *et al. Int. J. Syst. Bact.* **27**, 44; 1977). In particular, we now have information on 16S (18S) rRNA from a fairly large range of organisms.

Although initial results using this method tended to confirm the dichotomy of living organisms, a major surprise has

arisen from the analysis of a relatively obscure group of organisms—the methane-producing or methanogenic bacteria (Fox *et al. Proc. natn. Acad. Sci. U.S.A.* **74**, 4537; 1977). These strictly anaerobic bacteria have in common the ability to produce methane from CO₂ and H₂ and to obtain their energy by means of this pathway. They have proved to be a difficult group of bacteria to obtain in pure culture, but there has been an increase in research into them in recent years spurred on by their possible use in energy conversion from waste organic matter. They have been found to be a fairly diverse group morphologically, comprising both Gram-positive and Gram-negative bacteria. Indeed, they have often been considered as a heterogeneous collection of organisms which happened to have a common metabolic property—the ability to produce methane from CO₂ and H₂. However, a study of 16S rRNA from 10 species available in pure culture has shown that they rather represent a closely-related phylogenetic grouping which is quite distinct from all other bacteria so far analysed in this way. A measure of their distinctiveness is that two very different prokaryotes, the blue-green algae (bacteria) and the enteric bacteria, are much more closely related to each other than either are to the methanogenic bacteria. Further, the methanogens seem no more related to other bacteria than they are to the eukaryotic component represented by 80S ribosomes. Is it too much to suggest that this difference represents two quite distinct ancestral prokaryotic groupings? It may be too early to say definitely in view of our restricted information on methanogens, but there is some further contributory evidence. Quite apart from metabolic factors related to the ability to synthesise methane and their strictly anaerobic existence, methanogens contain no peptidoglycan in their cell wall, the pattern of base modification in their 16S rRNA is, for the most part, different from that in other bacteria and their tRNA lacks the so-called common sequence TΨGC present in all other organisms tested, be they prokaryotes or eukaryotes (quoted in Fox *et al. Proc. natn. Acad. Sci. U.S.A.* **74**, 4537; 1977).

Arising from this work, it has been suggested by Woese and Fox (*Proc. natn. Acad. Sci. U.S.A.* **74**, 5088; 1977) that there are three aboriginal lines of descent represented by three 'urkingdoms' or 'primary kingdoms'.

The methanogenic bacteria.

This group may have developed at an early stage in the evolution of the

Earth when there was an anaerobic atmosphere rich in CO₂ and H₂ (3 to 4 billion years ago) and they may have played a very important part in the transformation of the environment during this period. Consequently, the name suggested for this urkingdom is the archaeobacteria.

All other prokaryotes so far characterised. This group has three major divisions—the blue-green algae (bacteria), the Gram-positive bacteria and the Gram-negative bacteria. This urkingdom is called the eubacteria and it is the kingdom to which the chloroplast progenitor is derived. Unfortunately, no data seem to be available on mitochondrial rRNA. The cytoplasmic component of eukaryotes. This urkingdom, for which the name suggested is the urkaryotes, presumably represents the engulfing species of the composite eukaryotic cell. If it is granted that these are the three major lines of descent and if later evidence supports this view, what is the relationship between these lines of roughly similar levels of organisation? Do they have a common ancestor no longer represented by organisms on this planet? Little is known about the likely age of the eukaryotic cell, but there is some evidence that the prokaryotic cell types have been reasonably stable for at least three billion years (Shopf *Exobiology—Frontiers of Biology* **23**, 16; 1972). If all three urkingdoms did have a common ancestor, represented by a simpler cell structure than the prokaryote, the evolution of this structure into our major cell lines must have occurred within a relatively short time; during this period the structure of substances such as rRNA must have been very much more labile than they have been in the longer period that has followed. It is clear that the recognition of these three lines of descent represents an exciting phase in the history of biology and there remains much to be done in clarifying the position. □

The trouble with kinase crystals

from C. C. F. Blake

A COMMON question asked of X-ray crystallographers is: can you be certain that the structure of a protein in the crystal is the same as that in solution? Although no blanket answer can be given, the indications are that in most cases the crystalline structure is very similar to that in solution. However, as Koshland pointed out many years ago, protein structures are intrinsically finely balanced so that in the presence of ligands different structural states are taken up that are essential for activity.

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So a more searching question to ask is: can you be certain that in the presence of ligands, the protein in the crystal is free to take up its appropriate structural form? We can be much less sanguine about this question than about the first, and indeed it is becoming clear that in some cases it may be difficult to answer it at all. In the classic example of the oxygenation of deoxyhaemoglobin crystals, the dissolution of the original crystals and their replacement by new crystals of oxyhaemoglobin indicates in this case that the energy of ligand interaction is greater than the lattice energy of the crystals. But the energy balance need not always be this way round, and what happens when the lattice energy predominates is illustrated by some recent observations on kinases.

The ability of kinases to catalyse the transfer of the phosphoryl groups of ATP to their specific substrates with great efficiency, without at the same time phosphorylating water to any significant extent, has always been intriguing. Especially so since the X-ray studies of hexokinase and phosphoglycerate kinase have shown that ATP is bound at fairly exposed sites on the surface of the enzyme. It seems probable that ATPase activity is avoided by the induction of extensive conformational changes by the enzyme's specific substrates that result in a catalytically active enzyme. On this basis it is the inability of water to effect these changes that prevents its participation in the enzymic reaction. Not unexpectedly, then, X-ray studies of substrate binding to kinases have come rather hard up against the problem of the extent to which conformational freedom of a protein molecule is limited by the crystal lattice.

The study of glucose and nucleotide binding to yeast hexokinase by Steitz and his colleagues (*J. biol. Chem.* **252**, 4494; 1977) is a good example of what can happen. Leaving aside for the present nucleotide binding to the inter-subunit allosteric site, and concentrating on binding to the active site, the observations are as follows. Glucose and *o*-toluoylglucosamine bind to hexokinase at the same site in either its monomeric BIII or the dimeric BII crystal forms, producing similar extensive alterations in the protein structure. Nucleotide binding is more complex: the ATP analogue, adenylyl-5'- γ -imidodiphosphate (AMP.PNP) binds only at the allosteric site but not at the active site in the BII crystals, and in the BIII form only AMP, but not ATP, ADP or AMP.PNP, binds in the active site region. Since inhibitors of hexokinase with respect to ATP also bind at the AMP site it seems probable that this is the nucleotide

location in the active site. In addition building ATP into the AMP site places the γ -phosphoryl near the 6-hydroxyl of the bound glucose. In attempting to understand why ATP or its analogue do not bind at this site, Steitz argues that it is probably because it induces further conformational changes that cannot be accommodated by the crystal lattice. Thus by not permitting the conformational changes the crystal forces inhibit ligand binding. As Steitz points out, inability to bind ligands does not necessarily indicate irrelevant or inactive protein structures, but rather crystals unable to accept the conformational changes that the molecule must undergo during its catalytic cycle. A somewhat similar picture is obtained from substrate binding to the homologous yeast and horse muscle phosphoglycerate kinases, except here it is the phosphoglycerate substrates that are difficult to bind.

A more detailed view of essentially the same problem has come from work on adenylate kinase by Schulz and his colleagues (Sachsenheimer & Schulz *J. molec. Biol.* **114**, 23; 1977; Pai *et al. J. molec. Biol.* **114**, 37; 1977). The pig-muscle enzyme crystallises in three forms whose interconversion is pH dependent Form A, from which the enzyme structure was originally deter-

mined, revealed only one of the two expected nucleotide binding sites. However in Form B that has now been solved, the enzyme has undergone a conformational change, probably triggered by the ionisation of a histidine residue that opens a large pocket that represents the second nucleotide binding site. This site is missing in the A form because the reverse conformational change closes off its entrance. Schulz has been able to identify the new site with AMP binding, and the site known from the A form with ATP binding. With the new crystal form the way now seems open to examine the substrate complexes of adenylate kinase and get closer to the catalytic activity.

The significance of these two studies, not only for kinases but for other enzymes as well, is that the usual techniques of diffusing substrates into previously grown native crystals may be inadequate or even misleading if large conformational changes are intrinsic to the enzyme reaction. The growth of crystals of enzyme substrate complexes, by allowing a new crystal lattice to form around the conformationally changed protein rather than putting the two factors into opposition, seems a more appropriate way of defining the structural forms attained during the catalytic cycle. □

Fibroblast surface protein

from R. C. Hughes

A Conference on Fibroblast Surface Protein was held by the New York Academy of Sciences on November 30–December 2, 1977 under the chairmanship of A. Vaheri, E. Ruoslahti and D. Mosher. The proceedings are to be published in the *Annals of the New York Academy of Sciences* in 1978.

THE cold insoluble globulin (Cig), little regarded when it was first isolated from human plasma in the 1940s, has been the subject of an explosive growth of interest in the past few years under its aliases LETS (large external transformation sensitive protein), fibronectin, fibroblast surface antigen, cell surface protein (CSP), 250 K glycoprotein and cell adhesion factor amongst others. In these guises it is ubiquitous at the cell surface and in the extracellular matrix, and the interest arises because of its possible role in malignancy; on transformation LETS largely disappears from the cell surface. Since no agreement on nomenclature resulted from the meeting I shall arbitrarily use

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LETS to describe the protein associated with the cell in a relatively intimate or fixed state and Cig for the circulating form.

Biochemical characterisation of LETS and Cig show them to be dimeric glycoproteins with carbohydrate content of around 5% and molecular weight of around 450 K; they consist of two equally-sized polypeptide chains linked by several disulphide bonds. The amino acid composition of both proteins in various species is very similar. But there is a striking difference in the solubility of LETS and Cig in urea which remains unexplained, although it may be due to the association of LETS with cell microfilaments.

More direct comparison by peptide mapping of lactoperoxidase-labelled LETS from human, chick and hamster fibroblasts indicates incomplete homology (R. C. Hughes, National Institute for Medical Research Mill Hill) and P. Bornstein and his colleagues (University of Washington, Seattle) have found several differences in the cyanogen bromide fragments of human Cig and human LETS. On reduction Cig produces a subunit that migrated more rapidly on SDS-polyacrylamide gel electrophoresis than those of cellular

LETs, indicating a molecular weight difference of ~10,000–20,000. By contrast, M. Vuento (City of Hope Medical Center, Duarte) finds that human Cig and LETs subunits have very similar molecular weights, and that their tryptic and cyanogen bromide peptide fragments are indistinguishable. Vuento isolated his LETs from spent culture medium and there are indications that in this case the LETs glycoprotein might have been degraded during 'shedding' from the cell surface before isolation. If this is so the differences reported by Bornstein may relate to a piece of LETs polypeptide not present on Cig or on LETs isolated from spent medium.

Whether Cig and LETs represent identical gene products perhaps with some post-translational modifications, for example by limited proteolysis or carbohydrate additions, remains unresolved. Although no hard evidence was presented to the contrary formal proof will require more structural data, particularly peptide sequencing.

Apart from questions of structure the role of Cig in forming pathological 'cryofibrinogen' precipitates was also of interest. Cig and fibrinogen produce complexes which can be precipitated at low temperature by thrombin. Cig and fibrinogen are substrates for plasma transglutaminase—factor XII (D. Mosher, University of Wisconsin, Madison) which stabilises the precipitated complexes. M. Matsuda (Jichi Medical School, Tochigi-Ken) has found low levels of circulating Cig in the disseminated intravascular coagulation syndrome, presumably due to its depletion by complexing with fibrinogen and subsequent fibrinolysis.

Cig and LETs also bind to various forms of collagen and many complex functions are believed to derive from this affinity. Cig and LETs bind poorly to native collagen but much more avidly to heat-denatured collagen (E. Ruoslahti and E. Engvall, City of Hope Medical Center). The binding site is located within a 35-amino acid fragment of the collagen $\alpha_1(1)$ chain (H. Kleinman, National Institute of Dental Research, Bethesda) and seems to be different from that recognised by platelets. Neither Cig nor LETs seems to be required for cell attachment to native collagen (F. Grinnell, University of Texas) which agrees with the results of Ruoslahti and Engvall. *In vitro*, cell attachment and spreading on dishes coated with dried or denatured collagen is enhanced by LETs from several species and by human Cig (E. Pearlstein, New York University Medical Center, and Grinnell). Presumably Cig or LETs promotes adhesion of cells to collagen *in vivo* in areas such as damaged epithelium or possibly in base-

ment membrane, whose collagens are low in triple helical regions.

The association between LETs and collagen *in vivo* is still very unclear. LETs-like antigens have been identified in basement membranes but cellular contamination must be considered as a possible source and its absence in highly purified glomerular kidney membranes (B. A. Bray, Columbia University, New York) may argue for a rather superficial integration into the collagen superstructure. On the other hand, fluorescent-antibody-staining seems to show some correlation between collagen and LETs fibrils in the extracellular matrix and at the cell surface. The pericellular distribution of LETs and collagen is destroyed by trypsin, but LETs distribution is unaffected by collagenase, indicating some independence of collagen and LETs organisation at the cell surface (A. Vaheri, University of Helsinki).

An important site of LETs-enriched pericellular material is at points of cell

contact, even in virus-transformed cells (L. B. Chen, Cold Spring Harbor Laboratory) which in general are poor in surface LETs due to reduced rates of LETs synthesis (K. Yamada, National Institutes of Health, Bethesda). Direct evidence for a role in intercellular adhesion was also described by Yamada who has inhibited attachment of single BHK cells to cell monolayers by relatively high concentrations of dimeric chick LETs. Yamada and R. Hynes (Massachusetts Institute of Technology, Cambridge) described reversion of transformed cells to a more normal well-spread morphology on substrates by exogenous LETs, showing that some transformed cells can utilise LETs if supplied with it. According to Hynes, Cig is two to three times less active than LETs extracted from cells in promoting these effects which perhaps argues for structural differences or alterations in the plasma component and brings us full circle. □

Consciousness and the physical world

from Godfrey Vesey

A conference on Consciousness and the Physical World was held at the University of Cambridge on 9–10, January, 1978. It was organised by B. D. Josephson and V. S. Ramachandran, University of Cambridge.

As a rule scientists leave questions about the mind and its place in nature to philosophers. But there are exceptions to the rule. Some readers of *Nature* (168, 53; 1951) may remember J. C. Eccles suggesting explanations of how 'mind influences' might have an effect on the cerebral cortex. His speculations embraced psychokinesis and ESP. 'If the so-called psi-capacities exist, they provide evidence of slight and irregular effects which are precisely of the nature that must be postulated for brain-mind liaison.' Now there has been a whole conference on this and related issues. How much has changed in the attitude of scientists to these questions in the 27 years since Eccles' paper was published?

In general there is an increasing recognition by scientists of the need to think twice about questions involving 'the mind' before setting about answering them. Grammatically they may look like questions calling for an answer in terms of a postulated causal relationship of some sort. But the grammatical appearance

may be misleading. Some of the key words in which debate about 'the relation of body and mind' is conducted are 'I', 'consciousness' and 'feeling'. It is increasingly recognised that to talk about the meaning of such words may not be to talk about 'things', even things distinguished from the ordinary run of things by being described as 'private'. The first task is that of reaching agreement on what these words mean, how they are actually used; and of dispelling the inapplicable models of their meaning we have inherited from the past.

This general observation may be illustrated with some examples from the Cambridge conference. In his opening remarks H. C. Longuet-Higgins (University of Sussex) spoke of the elusiveness of the concept of consciousness, and stressed the importance of making sure that we used the word 'consciousness' in the same sense. He went on to distinguish several approaches to the concept. In connection with one of them, *via* the theory of observation, he remarked that it may be quite mistaken to regard consciousness as an *explicandum* of the same kind as a commonplace biological function such as feeding or reproduction. In connection with another approach he said something which could be taken as having a direct bearing on Eccles' attempt to discover what it might be about certain cortical states which render them susceptible to 'mind influences'. 'If one rejects the concept of a "homunculus" sitting at the controls of the brain, then

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the physical phenomena associated with consciousness need not be supposed to differ physically from any other observable phenomena.' But for a philosopher perhaps the most interesting thing about his talk was that he refrained from following a certain line of argument. He began a sentence 'When we use the first-person singular we are claiming . . .', then stopped short, and said 'No, let me start that again'. A possible continuation of the sentence he started would be: ' . . . awareness of something, a self, to which the word "I" refers'. Bertrand Russell once argued that we could not understand a sentence beginning with the first person singular pronoun 'unless we were acquainted with something which we call "I"' (Russell, *Problems of Philosophy*, Oxford University Press paperback, 28; 1967). Later he changed his mind, not being able to discover by introspection an 'I' performing an act of thinking (Russell, *Analysis of Mind*, Allen and Unwin, London, 17; 1921). The problem of the meaning of 'I' is one which has continued to exercise Cambridge philosophers. One of the best papers in my judgement is the recent 'The First Person', by G. E. M. Anscombe (In *Mind and Language*, (ed. Guttenplan) Clarendon Press, Oxford, 1975).

A number of speakers tried to find evolutionary explanations of our being conscious. According to H. B. Barlow (University of Cambridge) consciousness is one of nature's tricks to ensure that man is gregarious. In his argument for this conclusion he made a connection between consciousness and the ability to communicate. Roughly, consciousness is a matter of being able to represent things to oneself symbolically, and this ability is logically parasitic on the ability to represent things to others symbolically. A philosopher's reasoning for this might be that the behaviour of language-users is rule-governed, that if someone's behaviour is governed by a rule he must be to some degree conscious of the rule, and that it would not be possible to distinguish between correct and incorrect applications of a rule if there were not a community of language-users (Kenny *et al.* *The Development of Mind*, 94; Edinburgh University Press, 1973). Barlow went out of his way to express his conclusion as paradoxically as possible, by saying, for example, that we only become aware of a sensation when we are prepared to communicate it. Understandably, he encountered some scepticism.

In some respects the thesis of N. K. Humphrey (University of Cambridge) was precisely the opposite. According to Barlow self-awareness is parasitic on other-awareness (or, as he put it, 'the modelling of other people's behaviour is primary'); according to Humphrey other awareness is parasitic on self-awareness. The starting-point of Humphrey's argument was the view that an animal derives

concepts such as pain and hunger from its own internal feelings. A social animal then uses these concepts, acquired by introspection, to understand its fellow animals. This introspective consciousness 'has evolved as a biological adaptation for doing psychology in nature'. I was strongly reminded, by Humphrey's account of how concepts are acquired, of the doctrines of such empiricist philosophers as John Locke, J. S. Mill, Russell and A. J. Ayer. The doctrine that words have meaning by standing for experiences carries with it one of the most intractable problems of philosophy, the so-called 'Other Minds' problem: if 'pain' is a name I once gave to an experience I had, and proceeded to give to later experiences on account of their similarity to the original one, how, without giving it a different meaning, can I use the same word for what is in principle not experienceable by me, something in another mind? In his later works Wittgenstein argued that the empiricist doctrine about meaning is profoundly wrong. Someone who accepted his view and had to choose

between siding with Barlow or with Humphrey would choose to side with Barlow.

Eccles began his 1951 paper by remarking that 'for each of us only our own mind is an observable', and alluding to the argument that the private and restricted nature of the observation eliminates mind from the class of facts of experience upon which scientific investigation may properly operate: it is 'a field outside the matter-energy system of the natural world'. In my own contribution to the Cambridge conference I suggested that our treating people as people rather than as automata is a matter not of our believing them to have private access to supernatural processes but of our accepting some of the things they say, for example about their hopes and expectations, simply because they say them. The difficulty, as Wittgenstein once remarked, 'is not that of finding the solution but rather that of recognising as the solution something that looks as if it were only a preliminary to it' (Wittgenstein, *Zettel*, Blackwell, Oxford, 1967). □

Chromatin transcription probe

from R. S. Gilmour

OVER the past 15 years our ideas about gene regulation in animals have been influenced by observations on the *in vitro* transcription of isolated chromatin. The advent of complementary DNA (cDNA) has facilitated the analysis of chromatin transcripts for gene-specific RNA. However, a major drawback of this system is that endogenous *in-vivo*-made RNA which coisolates with the chromatin can also contaminate the isolated *in vitro* transcript. One method which seemed to offer a solution to this problem was to carry out the transcription in the presence of a mercury-substituted nucleoside triphosphate (usually Hg-UTP) and then to separate the *de novo* synthesised, mercurated RNA from non-mercurated endogenous RNA by affinity chromatography on thiol agarose columns. A number of groups purport to have shown the transcription of a specific gene in isolated chromatin in this way.

Recently, however, the validity of this approach and indeed of *in vitro* chromatin transcription in general has been questioned by new findings. In a recent issue of *Biochemistry* (16, 5135; 1977, see also *Biochem. biophys. Res. Commun.*, 75, 598; 1977), Zasloff and Felsenfeld suggest that the mercuration technique highlights a previously un-

suspected artefact of chromatin transcription. Briefly, these workers isolated *Escherichia coli* RNA polymerase transcripts of duck reticulocyte chromatin by incorporating Hg-UTP and analysed for globin RNA sequences by hybridisation of duck globin cDNA. Surprisingly, however, the synthesis of globin mRNA sequences was not sensitive to levels of actinomycin D which stopped virtually all other RNA synthesis from chromatin. The implication that the polymerase was in fact copying endogenous RNA rather than DNA was strengthened by the finding that when artificial chromatin templates were constructed from *E. coli* DNA and erythrocyte histones and deliberately contaminated with globin mRNA, this RNA was subsequently isolated with the purified mercurated transcripts. It would appear that when the polymerase copies endogenous RNA it forms a hybrid, one strand of which now contains Hg-UTP. The endogenous RNA however accompanies the newly synthesised mercurated RNA through the thiol agarose purification by virtue of its mercurated anti-strand and thereafter gives a spurious positive hybridisation. One obvious way round this is to heat the transcript before thiol agarose chromatography. The hybrid strands separate and endogenous RNA is no longer copurified with the transcript. When this is done, however, the transcript no longer hybridises to globin cDNA.

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An identical conclusion was reached by Giesecke *et al.* (*Nucleic Acids Res.* **4**, 3943; 1977) in their bid to detect the *in vitro* synthesis of ovalbumin in RNA sequences from chick oviduct chromatin with *E. coli* polymerase.

These reports are somewhat alarming since they would imply that no DNA-dependent gene-specific transcription can be detected in chromatin in which these genes are known to be active *in vivo*. How would this conclusion affect the interpretation of the vast bulk of chromatin transcription data accumulated over the past decade or so? Several comments spring to mind.

Before cDNA was available, chromatin transcripts were analysed qualitatively by hybridising purified *in vitro* labelled transcripts to denatured total DNA bound to nitrocellulose filters. The amount of label converted into RNase-resistant hybrid gave an estimate of sequence homology. In this analysis, transcription of endogenous RNA would give rise to a labelled copy which would hybridise to DNA in the same way as if correct DNA transcription had taken place except that the strand sense is reversed.

Gene-specific cDNAs have been made from ovalbumin, histone and a variety of globin messenger RNAs and used as hybridisation probes for chromatin transcripts. As already pointed out the data obtained are only valid where it can be shown that endogenous RNA sequences are not interfering with the analysis. Where this is so and it has not been considered necessary to resort to mercurated nucleotides it is difficult to see how gene-specific transcripts can arise other than by direct transcription of the DNA itself. However, a number of workers have indeed used the mercury technique, suggesting that endogenous RNA is a problem with chromatin in general. In this case the criticism of Zasloff and Felsenfeld is a valid one and unless steps are taken to avoid cross-contamination of transcripts with endogenous RNA the data cannot be sidered conclusive.

Attempts have been made to assess the fidelity of transcription from chromatin with respect to DNA strand selection by bacterial and isolated animal polymerases. In many cases where symmetrical transcription is seen this has been interpreted in terms of loss of enzyme specificity in *in vitro* conditions. However, endogenous RNA transcription rather than non-selective DNA strand copying might equally well account for the findings.

It should be mentioned that the strongest evidence for RNA copying presented by Zasloff and Felsenfeld and Giesecke *et al.* comes from experiments where purified mRNA is added to

The blind fishes of Persia

It was exciting to read the note by G. Thinès in *News and Views* (**271**, 305; 1978) which affirmed his 'great interest' in the discovery of two cavernicolous forms in the same well-like outlet. However, it is possible that a misunderstanding has occurred which needs to be corrected. The fish were indeed both found in the same well-like outlet; but this resurgence, which did resemble a well, was an entirely natural event. As much of the note's argument concerned the co-existence of cave forms in biotopes other than those of caves it is important to clarify the actual situation.

The two fish species were *Iranocypris typhlops* (Cyprinidae), first described by Bruun and Kaiser in *Dan. scient. Invest. Iran* **4**, 1-8 (1943, published 1950), and *Noemacheilus smithi* (Cobitidae), first described by P. H. Greenwood in *J. Zool.* (**180**, 129; 1976). The Danish paper, which also gives a photograph of the discovered location of *I. typhlops*, mentions that 'the water comes from the subterranean source in the background' of the picture. I discovered *N. smithi* by going to this precise spot and observing the blind loach in the same pool as the previously discovered blind carp. In his paper Greenwood confirms the Danish description and stresses the naturalness of this resurgence: 'There, exactly as described by Bruun & Kaiser, [Smith] found a small group of fishes swimming in the well-like outlet of a subterranean water-body. Since the habitat is fully exposed to light and in no way resembles a cave, it seems reasonable to suppose that the typically cavernicolous fishes living there are accidental strays from a true cave environment within the mountains.'

The case of *I. typhlops* coexisting with *N. smithi* is not similar to that of *Milyeringa veritas* and *Anommatophasma candidum*, or the other examples quoted by Thinès. Both *N. smithi* and *I. typhlops* have so far been found in only a single locality and that locality is neither a well nor an artificial outlet. It cannot be argued that their case is 'a typical instance of an already well-known

phenomenon, whose meaning for bio-speleological research is clear.'

What is still completely unclear is the nature of the true habitat of the blind fishes of Persia, so far discovered only in a single resurgence near Tang-e-Haft in the Zagros mountains. An attempt was made in 1977 by two caving experts equipped with breathing apparatus to gain access to the cave system. Greenwood and others considered that the 'well-like outlet' could not possibly be the sole habitat of the two Persian fishes. During the snow-melt period of spring this outlet always overflows to form a small stream, according to local villagers, but in May or June the overflow ceases. For the rest of each year the well of water has a surface area approximately 5 m by 3 m and this gradually decreases as the season progresses.

The two divers attempted their exploration in April when the outlet was no longer overflowing, it being an exceptionally dry year, and they descended to a depth of about 20 m. Right from the surface the resurgence narrowed until it became impossible for the men to progress further. Their opinion was that 'a major dry system did not necessarily exist within the mountain' and that 'a vast volume of water could be contained in a massive underground maze of flooded rifts' (personal communication). They thought it unlikely that anyone would find a way in to the real habitat of the Persian cave fishes as the nearby mountains 'were composed of limestone with very thin beddings, varying from 15 to 60 cms, which were almost vertical. Not only was the rock very friable, making major cave systems improbable, but any caves which did exist were likely to be high narrow rifts.' It would seem that, for the time being, the blind cave fishes of Persia are quite safe within their cave, save for the few of their number who stray inadvertently into a resurgence where they are (very occasionally) caught by passing naturalists.

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transcription systems. However, it is probable that endogenous RNA does not exist in this free form and may be tightly bound within the chromatin structure. While it is not known whether this RNA can be copied by RNA polymerase, any cytoplasmic mRNA contaminating the chromatin might well produce an artefact. The levels of cytoplasmic globin and oval-

bumin mRNAs in these systems are extremely high and therefore it is still not clear whether the observations are due to an intrinsic feature of the chromatin structure or inadvertent cytoplasmic contamination. Whatever the answer, the impact of these findings should encourage workers in this field to examine their own particular systems with ruthless scrutiny. □

I SHOULD like to comment on the article 'Advances in Chinese Restaurant Research' (*News and Views* 267, 756; 1977). Unfortunately your anonymous correspondent seems unaware of the background to a field which may have some superficially amusing characteristics but, in fact concerns some potentially serious issues.

The topic of the Chinese Restaurant Syndrome is hardly 'gripping' the Boston Medical Research Community. In fact, although it mildly interests most people, it 'grips' only those who get fairly severe symptoms. In trying to point out that the name of this Syndrome was unfortunate (since glutamate is used in other types of restaurants), I gave as examples, Japanese, Italian and French; I did not feel that I had to enumerate the names of all types of cuisine available in the Boston area. 'All-American' establishments are by no means 'blameless'; if by All-American food one means steaks, for instance, it has been found in some establishments that an 8 oz steak is not unlikely to be 'doctored' with 0.9 g of monosodium glutamate. As for the listing of what the correspondent found to be a disagreeably large number of symptoms, I will quote, by comparison, a recent description of yet another ill-defined, but quite real human malady: Symptoms of 'Classic migraine . . . in addition to the head pain . . . may also include photophobia, nausea, vomiting, constipation or diarrhea, weight gain and fluid retention followed by diuresis, scotomas or visual field defects, paresthesias or defects in motility, vertigo, and elevation of blood pressure.' (from Dalessio *Dietary Migraine*, *Am. Family Phys.* 6 No. 6, 60; 1972).

A large literature exists on the effects of glutamate in both animals and humans. An interest in the adverse effects, to (presumably) glutamate, both in animals and in some humans, has existed for a number of years. It is fairly well established that parenteral

Chinese Restaurant Syndrome

administration of glutamate causes vomiting in humans. Various studies, primarily since 1968, have indicated that the so-called Chinese Restaurant Syndrome is probably caused by the raised levels of free glutamate that are frequently added to such foods. This general problem is of both theoretical and practical interest. Many people (including many physicians) are unaware of so-called Chinese Restaurant Syndrome; people with fairly severe reactions to what is presumably monosodium glutamate, have been unnecessarily caused extended periods of anxiety (thinking that they had some neurological problem) because of this lack of general knowledge. A number of young children have been subjected to extensive neurological testing and have apparently been misdiagnosed and incorrectly treated because of a lack of awareness of 'glutamate overload' intolerance. (Reif-Lehrer & M. G. Stemmermann *New Engl. J. Med.* 293, 1204; 1975; Reif-Lehrer, *Pediatrics* 58, 771; 1976.) There is also an increasing literature on glutamic acid as a neuro-excitatory substance and this amino acid is currently being examined as a putative neurotransmitter in certain cells. Numerous investigators have reported that young rodents treated with glutamate are subject to both retinal and hypothalamic damage. Similar results have been obtained in the hypothalamus of monkeys, but are more controversial.

Animals which have been examined do respond to glutamate overloading with convulsions. A variety of other responses in animals have also been reported, and either glutamate or aspartate are routinely used to eliminate the B wave of the electroretinogram of isolated retinas, again indicating damage to the cells of the inner retina. Several studies in humans, as well as animals,

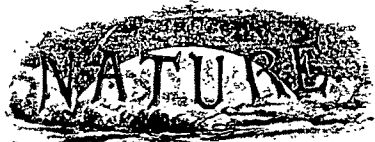
have shown that ingestion of raised levels of free glutamate does indeed lead to abnormally high blood levels of this amino acid.

In the course of some studies concerned with glutamate metabolism in the retina, I became interested in the more general topic of glutamate metabolism in humans and in particular in a question which had apparently remained unanswered in the literature: namely, how many people experience the strange phenomenon called Chinese Restaurant Syndrome, and what may be different about the metabolism of these sensitive individuals. The fact that this issue is of importance to others may be attested to by public hearings (July 25-26) before the FASEB Select Committee on GRAS Substances on Glutamates, which were held in Washington. A final report of this Committee should be ready early in 1978. The opinion expressed in the tentative FASEB report and confirmed at the Washington hearings was, that the issues concerning glutamate consumption are of importance and need further exploration. Such further exploration will of course, require time, money, appropriate expertise, and an interest on the part of the scientific community to explore the question without preconceived bias.

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Our correspondent replies: I am very sorry that Dr Reif-Lehrer mistook my light-hearted comments for an attack on research into the harmful effects of glutamate. What I did intend to question was the usefulness of accumulating a vast body of anecdotal reports, when they are not accompanied by parallel tests for the substance in question. This kind of approach makes it all too easy for the food industry to cry 'not proven'.



A hundred years ago

FROM the last report of Dr. Dohrn, the director, we notice that the zoological station at Naples has developed a most remarkable degree of activity, and is becoming a valuable centre of biological research. By the generosity of the Prussian Government it has been provided with a small steamer, and the uninterrupted expeditions in this vessel have secured to the laboratories an enormous and most varied stock of material for research. Dr. Dohrn has

carefully organised a plan for the systematic examination of the entire fauna of this part of the sea, to be accompanied by exhaustive description. The literary portion of the work will consist of elaborate monographs on all the families and species represented in the Gulf of Naples. They will not be prepared by the members of the station only, but it is hoped to procure the assistance of all familiar with this special department, and the contributions can be in English, French, German, or Italian. Two monographs on the Elenophorae and Balanoglossi will appear during the present year, and arrangements have been made for the speedy preparation of eleven others.

THE Radicals in the French Chamber cannot be accused of opposition to the claims of science. We notice that in a

late session a member of the extreme left proposed an amendment to the budget of instruction, which provided for the appropriation of 30,000 francs for an expedition to California to observe the next transit of Mercury, 40,000 for the continuation of the explorations in Northern Africa, where it has been proposed to admit water from the Mediterranean, and 100,000 to enable the Abbé Debès to make a journey across Africa from Zanzibar to the Congo. As the appropriation was granted, we may hope soon to see the latter portion of it cause the appearance of a new rival of Stanley, for the Abbé has had, like Livingstone, invaluable experiences as a missionary, which will enable him to enter upon the undertaking with great promises of success.

From *Nature* 17, 21 Feb, 329; 1878.

review article

Repair deficient human disorders and cancer

R. B. Setlow*

The analysis of the repair of damage to DNA in mammalian cells leads not only to a knowledge of which environmental agents are deleterious to living creatures, but also to an understanding of which reaction products in DNA are potentially carcinogenic and which tissues are the more sensitive.

In a number of recessively inherited human disorders, the affected individuals are cancer prone¹. The prevalence of cancer among such individuals ranges from significantly higher to orders of magnitude higher than in the general population²⁻⁴. Three of these disorders—xeroderma pigmentosum (XP), ataxia telangiectasia (AT) and Fanconi's anaemia (FA)—are associated with defects in the ability of cells to repair certain kinds of physical or chemical damage to their DNA. The existence of such disorders is direct evidence that damage to DNA can be carcinogenic, and is the best available evidence for a causal association between mutagenic and carcinogenic agents—an association used extensively as an environmental detection screen for carcinogens⁵. The support for the causal connection is further strengthened by the observation that mutations occur in XP cells at lower doses of carcinogens than those affecting normal cells⁶.

The clinical symptoms of the disorders are completely different, as are the known defects in repair. XP is characterised by the extreme sensitivity of skin to sunlight^{3,4,7}; AT by progressive cerebellar ataxia and dilated blood vessels in the eye, as well as immune deficiencies⁸; and FA by haematological abnormalities that ultimately lead to death as a result of haemorrhage or haematological failure¹. FA may also include various skeletal deformities. The diseases are rare, approximately one in several hundred thousand births, but heterozygotes are estimated to be one in several hundred⁹. However, heterozygotes do not show indications of the disorders and are not cancer prone, except in FA for which there is evidence of a higher risk of leukaemia. It has been estimated that 5% of all individuals dying of acute leukaemia are FA heterozygotes⁹. Thus, although the diseases are not general public health problems, analysis of the molecular defects in them should elucidate the molecular nature of the changes responsible for killing, mutagenesis and carcinogenesis, and give estimates of the probability that such changes result in biological effects. Since human risks can only be assessed from data on molecules and animals extrapolated to humans, usually by means of scanty epidemiological data, it is important that we have an understanding of the molecular mechanisms involved so as to provide a theoretical base for the extrapolations. Such a long-range, seductive goal has led to a wide range of investigations on the association of repair defects with other human conditions, including ageing and high blood pressure.

Almost all our notions about the molecular nature of repair come from studies on bacterial systems because of the large number of genetically well defined repair-deficient mutants and the relative ease in analysing photochemical and molecular changes in them^{9,10}. The genetic analysis of repair in mammalian systems is based on the naturally occurring human

inherited diseases. The concepts are simple but the problems are complex because physical or chemical agents make more than one change in DNA and there is no reason to assume that the changes have large biological consequences in normal cells. If a particular product is repaired well in normal cells and not in sensitive ones, it may be that the product is bad for the latter but innocuous for the former. Moreover, if a product is not repaired in both normal and sensitive cells, one cannot assume without other information that it has any biological effect. For ultraviolet radiation there are experimental tricks that permit one to show that pyrimidine dimers alter the biological function of DNA and affect both sensitive and resistant cells, even though the latter are highly proficient in excising dimers. The trick is to use the fact that photoreactivating (PR) enzymes in the presence of light are able to break pyrimidine dimers into monomers without affecting any other known photoproduct⁹. Hence, if ultraviolet (UV) affects some biological function and the effect of ultraviolet is ameliorated by subsequent exposure to PR light, one can assume that the initial biological effect arose from dimers in DNA. Such a conclusion does not indicate the molecular mechanisms by which dimers produce their effects. They could be the induction of viruses, changes in transcription or inhibition of repair of other products. The identification of dimers as inactivating transforming DNA, killing and causing mutations in bacteria, transforming fish cells, and inhibiting DNA synthesis in human cells means that ultraviolet is the best understood model for environmental carcinogenesis^{9,11,12}.

Excision defective XP cells seem to be analogues of the *uvrAB* mutants of *E. coli*. However, details of excision in *E. coli* are not well known because even though the *uvrAB* endonuclease makes single-strand nicks in UV-irradiated DNA, one can easily calculate from published data¹³ that the number of nicks are many-fold fewer than the numbers of dimers. Hence, the action of the endonuclease *in vitro* seems to be on minor photoproducts in DNA. Similar conclusions are reached about most of the UV endonucleases that have been isolated from prokaryotic or eukaryotic systems. Exceptions are those isolated from *M. luteus* and from T4 phage infected *E. coli*^{9,10}. It is no wonder that the details of repair of UV damage in human cells, where in XP there are at least five complementation groups⁷, are not known.

Types of repair

The existence of cell strains sensitive to certain environmental agents does not imply that the sensitivity arises from a defect in repair unless one can show that the initial deleterious products occur in similar numbers in both sensitive and resistant cells. But this demonstration requires a knowledge—usually incomplete—of what the products are. Hence, the best way to assess the role of repair is to measure host cell reactivation—

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the ability of cells to affect the survival of viruses treated with chemical or physical agents outside the cell. Low survival is interpreted as a defect in repair. The technique is applicable to unknown types of damage and to unknown types of repair system.

The direct measurement of excision repair requires determination of loss from DNA of products that have biological consequences. It is this latter aspect of the problem that has led to the emphasis on pyrimidine dimers in DNA^{9,14}. In subsequent experiments one can use indirect measures of repair such as unscheduled DNA synthesis, repair replication or the photolysis of incorporated BUdR^{9,14}. These techniques can be used as measures of DNA repair even though they do not identify uniquely what change is repaired or the changes that are biologically important. However, defective repair may not be readily apparent because a product of little biological consequence may well be repaired whereas a minor product with important biological consequences is not well repaired. This seems to be the case for alkylation damage to DNA. Both normal and XP cells are able to remove readily *N*⁷-alkylGua, but only normal cells can do it for the minor product *O*⁶-alkylGua¹⁵. Nevertheless, the various measures of excision repair usually give similar results. In cells with a low level of excision repair for dimers, repair replication is not necessarily an adequate measure of dimer excision because, presumably, repair replication also detects repair of minor photoproducts.

At a mean lethal ultraviolet dose to excision-defective cells there are many dimers per cell⁹. Hence, cells have evolved effective ways of bypassing alterations to their DNA. In both bacterial and mammalian systems the DNA made on a damaged template is often smaller than that made on undamaged ones¹⁶. The small DNA is subsequently chased into pieces the size of the parent, and the process of chasing the small DNA into large is called post-replication repair. In a number of instances the size of the small DNA is compatible with the idea that pyrimidine dimers act as blocks to synthesis, and this notion is reinforced by the observation that PR treatment before the pulse label results in much larger DNA in bacterial, marsupial, and chicken cells¹⁶; in all of which PR illumination breaks dimers into monomers. In bacteria the new DNA acts as if it contains gaps and the gaps are filled in by exchanges between parental and daughter DNA. Post-replication repair is defective in *rec*⁻ and *lex*⁻ mutants of *E. coli* and in such cells susceptibility to mutation by UV radiation is also greatly reduced¹⁷. These and other arguments have led to the notion that at least a part of post-replication repair in *E. coli* is inducible by small doses and such doses induce a new protein, the so-called protein X¹⁷. The appearance of this new protein is associated with the fixation of mutations.

It is reasonable to carry over these ideas to mammalian cells but the molecular mechanism involved in post-replication repair in mammals is in dispute. In some experiments the newly synthesised DNA acts as if it contains gaps that are filled in during post-replication repair and the gaps were estimated to be about 1,000 nucleotides long¹⁶, although more recent estimates put the number closer to 200 (R. B. S. and E. Grist, unpublished). There seem to be comparatively few exchanges¹⁸ and the evidence for the existence of gaps in the newly replicated DNA comes only from experiments on the photolysis of DNA repaired in the presence of BUdR. Synthesis may take place by a replicative bypass mechanism^{19,20} without production of gaps and there is evidence that the gaps, if any, are not opposite the dimers in the parental DNA^{21,22}. Perhaps both modes of synthesis take place. Post-replication repair is defective in XP variants but it can be speeded up by pretreatment of cells with small doses of ultraviolet or *N*-acetoxy-AAF^{24,25}. The role, if any, of this enhanced repair is not clear. It takes place not only after split doses but also with single doses (R. B. S. and E. Grist, unpublished). The role of post-replication repair in mutagenesis can be identified in bacteria because it is possible to remove the lesions at known times by PR treatment¹⁷. Such a technique has not been developed for mammalian cells. It

is an intriguing observation that protease inhibitors that block the induced repair functions in *E. coli* also acts as inhibitors of tumour promotion²⁶.

Defective excision or post-replication repair does not seem to be involved in the formation of spontaneous sister chromatid exchanges since various XP cell lines show no significant differences from normal cells²⁷. However, treated, excision defective XP cells always show more sister chromatid exchanges independent of the repair capability of the cells for the major product of the chemical agent²⁷. Hence, the main chemical damage detected as affecting DNA is not necessarily that responsible for chromosomal effects, although for ultraviolet the PR of chromosome aberrations in marsupial cells indicates that pyrimidine dimers are important²⁸. In any event increases in chromosome aberrations are correlated with decreases in excision repair, although sister chromatid exchanges are not²⁹.

Xeroderma pigmentosum

Xeroderma pigmentosum is the most widely studied repair-deficient human disease⁷. Affected individuals develop light-induced skin cancer with a prevalence among whites at age 20, of approximately 100%—a value at least 10³-fold greater than for the general population³⁰. All XP cells are defective in at least one mode of repair of ultraviolet damage to DNA. Most XP individuals have a defect in the excision repair pathway for ultraviolet damage. The defect is not complete, but may be over 90% in some individuals and only 50% in others⁷. Although few quantitative comparisons of repair kinetics have been made by one laboratory using several methods, it is comforting that similar estimates of the extent of excision defects are found by a number of different techniques, such as cell survival and mutation after irradiation⁶, ability to reactivate ultraviolet inactivated viruses³¹, the loss of dimers from DNA¹⁴, loss of ultraviolet endonuclease sensitive sites³², unscheduled DNA synthesis⁷, and repair replication¹⁴ either by direct observation or by the photolysis of incorporated BUdR. Among the excision-defective individuals there is a good correlation between the severity of the molecular defect and the severity of the disease³³, although the latter also must depend on the exposure to the deleterious wavelengths in sunlight. The fact that the mutation frequency per unit dose is higher in these XP cells, but that the frequency at a given survival is the same for normals is a biological indication that excision repair is error-free⁶. Biochemical and biophysical data indicate also that excision repair makes no large errors³⁴.

The defect in excision seems to be associated with a malfunction of the incision step during incubation after UV irradiation of XP cells, since only small numbers of single strand breaks are observed compared with the numbers of dimers in DNA^{14,35}. Moreover, when T4 endonuclease is introduced into irradiated XP cells, unscheduled DNA synthesis approaching normal levels is observed and the introduced enzyme increases cell survival³⁶. Excision defective cells fall into five complementation groups⁷. Hybrids of any two are close to normal in unscheduled synthesis, repair replication and loss of sites sensitive to ultraviolet endonuclease, as well as by the ability of such heterokaryons to reactivate ultraviolet irradiated viruses. One might think that active endonuclease is formed in the heterokaryons from diffusible parts of inactive multimeric enzymes. However, sonicates of XP cells are able to excise dimers from exogenous DNA but are not able to excise dimers from their own chromatin, whereas normal cells are³⁷. Thus, the nature of the molecular defect in XP cells is not known.

A sixth group of xeroderma pigmentosum individuals is proficient in excision repair. The existence of these individuals was an important exception to the association between the severity of repair defect and the severity of the disorder. However, cells of this group are now known to be defective in post-replication repair²³, are more sensitive to ultraviolet radiation⁶, and are not fully proficient in host cell reactivation³⁸. More-

over, not only the mutation frequency at a given dose, but the mutation frequency at a given survival is higher than that of normal cells, implying that some steps in repair of XP variant cells are more error-prone than in normal or in conventional XP cells³⁹.

The experiments summarised above use acute exposures of cells to 254 nm radiation at doses of 1–20 J m⁻². People receive much smaller equivalent doses per exposure. One minute exposure to the noon sun in Texas in September would be equivalent to approximately 0.1 J m⁻² of 254 nm⁴⁰ and the amount transmitted through the upper inert layer of skin would be closer to 0.01 J m⁻². Hence, the actual carcinogenic environment is a far cry from the experiments done in the laboratory. Moreover people are exposed to strong visible light at the same time that they are exposed to ultraviolet. Human cells have PR enzyme activity and XP cells in culture have less activity than do normals⁴¹. Although the enzymatic activity of cells in culture depends upon the culture medium⁴², it is reasonable to suppose that the enzyme would act *in vivo* and that the number of dimers in cells would be determined by the relative levels of ultraviolet and visible radiation and the PR and excision enzymes. It is intriguing that in all the XP cell lines investigated the sum of the three repair activities: excision, post-replication, and PR is about one half of normal⁴³.

The fact that all XP cell lines are defective in one or more repair pathway for UV damage makes it attractive to speculate²⁴ that UV-induced skin carcinogenesis in normal human-beings has a low probability because most of the lesions are removed and DNA synthesis beyond any that remain is relatively error free. In excision defective XP cells many dimers remain and replication beyond them, although relatively error free, makes an appreciable number of mistakes and hence gives a high possibility for neoplastic transformation. In the XP variants there are a few more dimers than in normals because of a defect in PR, but replication beyond dimers is error prone and, as in the case of conventional XPs, an appreciable number of mistakes is made. Similar speculations have been made for chemical damage⁴³.

From ultraviolet to chemicals

Bacteria that are defective in repairing ultraviolet damage are also defective in repairing a number of other damages to DNA, for example, nitrogen mustard, 4-NQO, and the effects of black-light plus psoralen^{9,44}. Hence it is not surprising that the same is true for XP cells, and the repair of damaged DNA is more general than just the special case of repair of pyrimidine dimers. There are several damaging agents that are repaired effectively in XP cells⁴⁵⁻⁴⁸. Such damage results from ionising radiation, the photolysis of BUdR and the action of simple alkylating agents. Repair in these cases involves both the repair of single strand breaks and probably excision repair mediated by *N*-glycosidases¹⁰ or by spontaneous base removal followed by the action of an apurinic endonuclease¹⁰. However, apurinic endonuclease isolated from XP cells shows changes when compared with the normal enzyme⁴⁹. The classification of chemical agents as making repairable or irreparable damage in XP cells is useful but not unique because a number of different products may be formed from a single agent. For example, alkylation of DNA affects many groups. The *N*⁷-alkylGua is removed efficiently in XP cells but the *O*⁶-alkylGua is not⁴⁵. Table 1 lists agents and products in terms of their repair by XP, AT, and FA cells. In the small number of cases that have been investigated carefully the sizes of the patches for agents repaired effectively by normal cells, but not by XP cells have been measured. The patches are large (30–100 nucleotides) and they are an order of magnitude larger than the number of bases inserted during repair of single strand breaks⁴⁶⁻⁴⁸.

The simplicity of the above picture is deceptive. One might suppose that the compounds mimicking ultraviolet would be acted upon by common repair pathways since the rate-limiting step in their repair is considered to be the initial endonucleolysis

(see above). However, acetylaminofluorene (AAF) and ultraviolet-induced damage do not compete for this putative common step. AAF treatment of normal human cells does not inhibit dimer excision at ultraviolet doses that saturate the excision pathway⁶⁹. Thus, our molecular picture of excision is far from complete. Nevertheless, agents that mimic the ultraviolet effect of excision also mimic its effects on cell survival and mutagenesis^{6,7} and in post replication repair. Moreover, in XP variants post-replication repair after ultraviolet or AAF is enhanced by treatment of the cells several hours earlier with small doses of either ultraviolet or AAF^{24,25}.

Repair in transformed or differentiated cells

As a general rule proficiency in repair of physical or chemical damage to DNA is independent of the cell type or tissue of origin. For example, transformation of cells by SV40 virus does not affect the ability or inability to repair ultraviolet damage⁷ and all tissues tested from XP individuals^{3,70}, including melanoma cells, are as defective in repair as are fibroblasts or lymphocytes. Moreover, tissues from non-XP individuals repair ultraviolet damage whether the tissues represent material from spontaneous tumours or normal tissue⁷⁰. Nevertheless, repair does not seem to be uniformly distributed over the chromosomes or throughout chromatin^{71,72}.

There are important exceptions to the above generalisation: (1) lymphocytes stimulated by concanavalin A are 10-fold more proficient in unscheduled DNA synthesis after AAF treatment than are unstimulated lymphocytes⁷³. It was concluded that the rate of excision is a function of the capacity for DNA synthesis and that unstimulated lymphocytes cannot readily be used to distinguish repair arising from agents that give rise to long patch repair from those that give rise to short patch repair. (2) Some melanoma cells are very resistant in culture and resistance is not associated with a lower yield of ultraviolet induced dimers nor with an increase in the rates of excision or post-replication repair⁷⁴. (3) Mouse fibroblasts are defective in excision repair of ultraviolet damage^{48,75} and so are epithelial cells in mouse skin⁷⁶. (The interpretation in an earlier report⁷⁷ of excision of mouse skin is confounded by the high surface, but low average, dose of 254 nm.) However, early passage cells from 13–15-d-old mouse embryos excise dimers from DNA into the acid soluble fraction of the nucleus⁷⁸. Cells from passages greater than 7 cannot do this nor can early passage cells from 17–19-d-old embryos. The latter are like cells from adult animals or most rodent cells in culture in that they do little excision repair of pyrimidine dimers. Similarly, after UV irradiation myocardial cells from newborn rats show extensive unscheduled DNA synthesis compared with cells from 3–12-month-old animals⁷⁹. These findings raise the possibility that the mutations leading to the XP phenotype are not in structural genes for proteins involved in excision repair, but are alterations in control elements that result in shutting off portions of repair pathways.

Ataxia telangiectasia

The association between exposure to sunlight and high prevalence of skin cancer in XP made it relatively easy to relate the disease to defects in repair of ultraviolet damage, especially since there were good hints from prokaryotes that pyrimidine dimers were typical lesions to be investigated. AT is more difficult to understand at the molecular level because cancer in this disease has not been associated with a particular environmental agent. The realisation that AT individuals are sensitive to X rays (see ref. 60), and that their levels of spontaneous and radiation induced chromosome aberrations are increased⁸⁰ led to the demonstration that fibroblasts from affected individuals are 3–4-fold more sensitive to X rays than normal cells⁶⁰. AT cells are proficient in the repair of ultraviolet^{83,84} and AAF damage⁸⁵. The main biological effects of X rays are thought to be base damage—perhaps double-strand breaks⁹.

All the AT cell lines investigated are proficient in the repair of single- and double-strand breaks^{60,61}. However, 6 out of 12 lines are defective in repair replication following anoxic irradiation⁶². (Anoxic radiation enhances the proportion of base damage to single-strand breaks.) Four of the cell lines showed normal repair replication. Hence, AT is genetically heterogeneous as is XP. Among three of the repair defective cell lines, fusion studies showed that there were two complementation groups⁶².

Table 1 Damaging agents or products for which cells are repair proficient or deficient

Xeroderma pigmentosum	
Proficient	Deficient
	Ultraviolet
	—dimers
	—protein DNA links ⁶⁰
	—strand breaks ⁶¹
Ionising radiation	Ionising radiation—anoxic
—strand breaks	4—NQO
—anoxic	AAF damage
4—NQO (minor comp)	ICR—170
NO—carbaryl ⁶²	Aflatoxin
NO—Me guanidine	K—region epoxides ⁶
MNNG	Br benzanthrane
MMS	Br ₂ Me benzanthrane ⁶³
MNU	EMS
EMS	O ⁶ -alkylGua
N ⁷ -alkylGua	Psoralen + light ⁶⁵
Proflavin + light ⁶⁴	Chlorpromazine + light ⁶⁶
	BCNU
	HNO ₂ ⁶⁷
	Propane sulfone
Mitomycin C ⁶³	Decarbamyl mito C ⁶³
Ataxia telangiectasia	
Proficient	Deficient
	Ionising radiation
Ionising radiation	—chromosomes ⁶⁹
—strand breaks ^{60,61}	—survival ⁶⁰
—endonuclease sites ⁶²	—endonuclease sites ⁶²
	Actinomycin D ⁶³
Mitomycin C ⁶³	Mitomycin C
MMS ⁶³	MMS
Ultraviolet ⁶⁴	
AAF—damage ⁶⁵	
Fanconi's anaemia ⁶⁶	
Proficient	Deficient
Decarbamyl mito C	Mitomycin C
MNNG	Psoralen + light
4—NQO	HN ₂
MMS	DNA crosslinks
	γ-rays ⁶⁷
Ultraviolet	Ultraviolet (high dose) ⁶⁸

Cells are categorised on the basis of survival, host cell reactivation, or by chromosomal, biochemical or biophysical measures of repair. (A listing in both categories means that there are several products of an agent or that all cell lines are not the same. In addition to specific references consult the text and refs 7, 14, 45–48.)

A protein extract from *M. luteus* makes single-strand breaks in DNA X ray-irradiated *in vivo* or *in vitro*. The extract can be used to determine whether such nuclease sensitive sites are removed from DNA during incubation following irradiation. Two cell lines defective in repair replication were assessed by this technique and were found to remove endonuclease sensitive sites at a much slower rate than normal cells⁶². The nature of the alteration that is not excised is unknown. It could be thymidine damage of the hydroxy hydroperoxide type even though such damage is removed readily from exogenous DNA by cell sonicates of repair defective cells⁶⁰ because, as in the case of pyrimidine dimers and XP sonicates³⁷, such sonicates might not mimic conditions *in vivo*.

The repair defects in AT are not confined to X rays but include chemical damages as well (see Table 1). Hence, there is a strong possibility of identifying the molecular nature of some

of the damages repaired poorly in AT and perhaps estimating whether there are particular environmental hazards associated with the initiation of cancer in this disease.

Fanconi's anaemia

A genetic analysis indicates that the defect is heterogeneous⁸¹. Cells from affected individuals show more spontaneous chromosome aberrations than do normal ones⁵⁹, and treatment of cells with DNA cross-linking agents such as nitrogen mustard, mitomycin C, or psoralen plus black light gives rise to 30-fold more chromosomal aberrations than observed in treated normal cells⁶⁶. Monofunctional agents do not give rise to such a big difference although the number resulting from UV irradiation is approximately fivefold more than in normal cells⁶⁶. These data are interpreted as indicating that FA cells have a defect in the repair of cross links⁶⁸. On the other hand treatment with mitomycin C results in a decrease in the numbers of sister chromatid exchanges⁸².

FA cells are appreciably more sensitive to killing by mitomycin C than are HeLa or XP cells and their DNA, analysed on alkaline gradients immediately after treatment, sediments more rapidly than DNA from untreated cells⁶⁸. At 4 h after treatment more of the DNA is larger than immediately after treatment. These data indicate that cross linking reactions take place after treatment. In normal or XP cells treated with mitomycin C a decrease in molecular weight is observed immediately after treatment, and in several hours the size of the DNA from treated cells returns to normal. The pattern of changes is as if an endonuclease breaks the affected DNA and repair is completed by excision, polymerisation and ligation. The repair of mitomycin C damage seems to be by pathways different from that of ultraviolet damage since cells that repair such damage show few strand breaks during repair^{14,35}.

FA cells may also be slightly defective in the repair of γ-ray and ultraviolet damage. γ-irradiated adenovirus does not grow as well on FA cells and, at high doses, ultraviolet irradiated virus does not grow as well as on normals⁶⁷. At high doses, but not low, the excision repair of γ-ray products or of pyrimidine dimers seems defective⁶⁸. However, as for all cells investigated to date, the repair of single strand breaks is not deficient, but in cell homogenates, the ability to remove high doses of t⁺ from exogenous DNA is defective in two of four cell lines investigated⁸³.

Bloom's syndrome

A repair deficiency is inferred from the finding of large numbers of spontaneous chromosomal aberrations and well over a 10-fold increase in sister chromatid exchanges in untreated lymphocytes^{1,59,84}. Lymphocytes from some individuals have a bimodal distribution of the sister chromatid exchanges⁸⁴. This finding suggests that the syndrome results from changes at a regulatory rather than a structural locus. The dimorphism is not observed in fibroblast or bone marrow cells. The chromosomes are more sensitive to radiation⁵⁹ and the cells are slightly more sensitive to ultraviolet⁸⁵. The rate of fork motion during DNA replication is slower than normal in Bloom's syndrome⁸⁶ and might account for the observation that the sedimentation of pulse labelled DNA after UV irradiation is somewhat slower than in normal cells⁸⁵.

Other human diseases and conditions

The successes in correlating some diseases causing cancer proneness with defects in DNA repair have led to searches for other repair deficient diseases. Light sensitive diseases other than XP have not been found to be defective in excision of ultraviolet damage⁸⁷. However, individuals with actinic keratoses—a potential precarcinogenic condition—have slightly decreased levels of unscheduled synthesis⁸⁸, and high blood

pressure has been associated with a decrease in repair and an increase in chromosomal aberrations in lymphocytes treated with AAF⁸⁹. The cells of Cockayne's syndrome are reported to be ultraviolet sensitive, but the molecular defect is not known⁹⁰.

The screening of cells for X ray-sensitivity has indicated that cells of D-type retinoblastoma individuals are more X ray-sensitive than normals or those of familial retinoblastoma⁹¹. On the other hand fibroblasts from four individuals with abnormal responses to X rays have normal survival curves and normal repair of single strand breaks⁹².

Two general types of experiment have been done in attempts to correlate ageing with changes in DNA repair. In the first the repair capabilities of young and old cells in culture were compared and in the second cells with syndromes of premature ageing were investigated. Both gave somewhat equivocal results. Cells from some progeroid individuals were reported to be defective in the repair of single-strand breaks arising from ionising radiation⁹³, but other investigators have not been able to obtain these results^{94,95}, and chromosome breakage is not abnormally sensitive in these cells⁹⁶. However, measurements of the ability of γ -ray irradiated adenovirus to grow on progeroid cells indicates that they do have some deficiency⁹⁷.

Late passage cultures may or may not show reduced capacities to repair damages of various types⁹⁸⁻¹⁰⁰ but the reduction, at least for ultraviolet damage, is not uniformly distributed among cells¹⁰⁰. Cells that do not go through the cell cycle do not do appreciable unscheduled synthesis, but late passage cells that do cycle do repair. Hence, there is no evidence that a defect in repair leads to senescence. It might be a consequence of it. I interpret the intriguing correlation between DNA repair in ultraviolet irradiated cells in culture and the life span of the donor animal⁷⁵ not as indicating a casual relation between life span and repair, but a consequence of the turning off of parts of the repair system at early ages in the development of some animals.

Conclusion

Cells from most individuals are proficient in the repair of DNA damage. Retrospective studies have shown that individuals defective in repair are cancer prone and our knowledge of the molecular nature of the defects leads to the strong prediction that unrepaired damage to DNA has a high carcinogenic potential. The complexity of carcinogenesis, on the other hand, also indicates that not all cancers arise from defects in repair. Nevertheless, even in normal individuals the rate of DNA repair compared with other cellular processes should be an important parameter in the initial steps in carcinogenesis. Hence, an understanding of the control and kinetics of repair processes is as important as a knowledge of the activation and inactivation pathways for chemical carcinogens in extrapolations of carcinogenic hazards to humans.

This research was carried out at Brookhaven National Laboratory under the auspices of the United States Department of Energy.

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articles

Measurement of magnetic fields in a tokamak using laser light scattering

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Local magnetic fields in a tokamak plasma have been measured by laser light scattering from the free electrons. This technique exploits the sensitivity of the cyclotron modulation of the scattered light spectrum to the angle between the local magnetic field and the plane in which the scattered light is observed. Theoretical constraints for observation of the modulated light spectrum are satisfied in the experimental conditions. Sufficient accuracy is achieved to derive the detailed volume current distribution—an essential factor in the energy balance and in plasma stability. The method may be applied to the considerably larger fusion research facilities currently envisaged.

IN controlled fusion devices such as tokamaks with strong magnetic fields, the gross stability and equilibrium of the plasma are governed by the pitch angle of the force lines¹. The strong toroidal field, B_ϕ , combined with the relatively weak self field, B_θ , due to axially driven heating currents, as shown in Fig. 1a, can provide a stable configuration for the plasma. This paper reports on measurements of the distribution of field directions from which the poloidal field distribution, $B_\theta(r)$, can be calculated. The equilibrium and stability behaviour of the plasma can then be studied in terms of the stability factor¹ q ($q(r) = rB_\phi/RB_\theta$). Measurement of $B_\theta(r)$ allows the current density profile, $j(r)$, to be calculated from the B_θ field distribution which gives the local ohmic heating rate and effective charge, Z_{eff} .

Following the first successful measurements of the electron temperature distribution in tokamaks using laser beam scattering², we have tried to bring magnetic fields within the set of plasma parameters which can be measured by this technique, as it uniquely offers high spatial and temporal resolution without perturbing the plasma.

It has been shown theoretically^{3,4} that the magnetic field influences the scattered light spectrum only when the differential wave vector $\mathbf{k} = \mathbf{k}_s - \mathbf{k}_o$, Fig. 1b, is nearly perpendicular to the local magnetic field vector, \mathbf{B} . When this condition is satisfied the spectrum is frequency modulated with peaks separated by the electron cyclotron frequency, ω_{ce} . Here we exploit the sensitivity of the depth of modulation to the angle between \mathbf{k} and \mathbf{B} . Due to the way in which the ohmic currents $j(r)$ are distributed through the plasma, the magnetic fields and their pitch angle, ε illustrated in Fig. 1, vary with plasma radius. Numerically, $\varepsilon = \arcsin B_\theta/B_T$ where $\mathbf{B}_T = \mathbf{B}_\phi + \mathbf{B}_\theta$. In tokamaks, the toroidal field B_ϕ can be set with considerable accuracy ($\lesssim 1\%$) so that the pitch angle $\varepsilon(r)$ is the quantity of immediate interest.

Measurement of the radial variation in pitch angle in the present study is based on the detection of those particular scattering planes, that is, planes defined by the directions of

the incident and scattered light, which show frequency modulation of the spectrum.

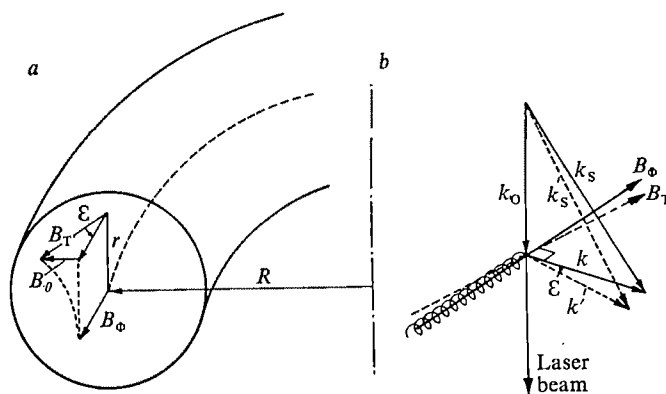
Unfortunately, in low density, $n_e \approx 10^{19} \text{ m}^{-3}$, high temperature, $T_e \approx 1 \text{ keV}$, plasmas as in tokamaks, the scattered light flux in the individual harmonics is too weak to be recorded directly. We overcame this difficulty by using light collection optics and a Fabry-Perot dispersion system which 'multiplexes' the harmonic content to a detectable level. The Fabry-Perot allows the angular information in the dispersed spectrum to be related to the variation of field line pitch angle with radius of the plasma column.

Other experimental studies of the location of magnetic surfaces in tokamaks have been reported recently. Alladio and Martone⁵ have determined a local current density by measurements of the displaced Gaussian scattered spectrum. In high temperature tokamaks, such a measurement is difficult because of the low ratio of the drift to thermal speed for the electrons, typically $v_d/v_e \approx 0.03$. Non-scattering methods include second harmonic generation at the upper hybrid frequency in the ST tokamak⁶ and the location of q surfaces by collimated neutral deuterium beams traversing the ATC tokamak⁷. Another technique by McCormick *et al.*⁸ involves the detection of σ and π components of the Zeeman-broadened emission lines from a neutral lithium beam which is directed through the plasma.

Theoretical background

When the scale length, k^{-1} , of the scattering geometry, as defined by the laser wavelength and scattering angle, is much less than the plasma Debye length, λ_D , only uncorrelated motions of the scattering electrons are represented in the

Fig. 1a, The magnetic field configuration in a tokamak. b, Vector diagram for scattering in a magnetic field. Full lines show situation where there is no B_θ field—on the magnetic axis. Dotted lines indicate how the wave vector plane rotates as B_θ assumes finite values.



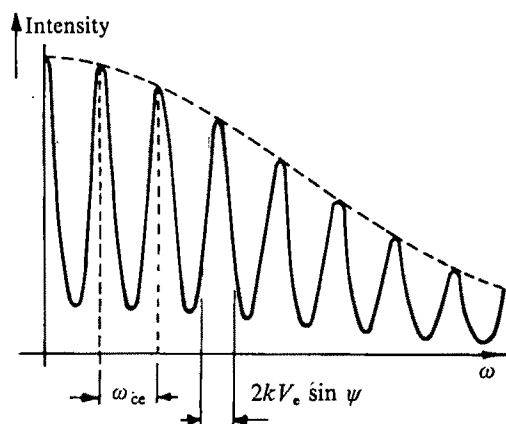


Fig. 2 Scattered spectrum for a magnetised plasma for \mathbf{k} almost perpendicular to \mathbf{B} .

Doppler broadened scattered spectrum. A single electron in the presence of a magnetic field will gyrate about a field line so that the velocity component in the \mathbf{k} direction will vary sinusoidally in time at the gyro frequency $\omega_{ce} = eB/m_e$. If the radius of gyration exceeds the scale length, the frequency modulated scattered spectrum will be a sequence of lines separated by the gyro frequency. The detailed spectrum of light scattered by an ensemble of electrons in a magnetised plasma has been calculated by Lehner and Pohl⁹:

$$S(k, \omega, \psi) = \frac{1}{\pi z^2 k v_e \sin \psi} \sum_{n=-\infty}^{+\infty} \exp(-n^2 z) \times \exp \left[- \left(\frac{\Delta \omega - n \omega_{ce}}{k v_e \sin \psi} \right)^2 \right]$$

where $z = k^2 r_L^2 \cos^2 \psi$ and ψ is the angular deviation from perpendicularity of the scattering vector \mathbf{k} to the field \mathbf{B} . It consists of a series of Gaussian peaks of width $2k v_e \sin \psi$, separated by the gyro frequency, Fig. 2. The peaks are bounded by a Gaussian of $2k v_e$ width, limiting their number to approximately $2k v_e / \omega_{ce}$ (or $2k r_L$, where r_L is the Larmor radius). Clearly, when $k \sin \psi v_e \simeq \omega_{ce}$ the modulation will disappear; this shows the critical dependence of the effect on the orthogonality angle ψ . A factor of 2 change in the ratio of these two quantities, that is, $k \sin \psi v_e / \omega_{ce}$, may cause the degree of modulation to change from about 90% to 15%. In the present experiment with a ruby laser the angle ψ has to be less than 0.5° for modulation to be observed. Other practical factors such as the finite acceptance cone of the scattered light and the variation in magnetic fields over the scattering volume will contribute to demodulation¹⁰.

Experimental background

Experimental evidence of modulated scattered light spectra from relatively dense ($n_e \simeq 10^{22} \text{ m}^{-3}$) plasma has been reported by Evans and Carolan¹¹, Kellerer¹², and Ludwig and Mahn¹³ who succeeded in resolving individual gyro peaks. However, in tokamak discharges with typical densities of $n_e = 10^{19} \text{ m}^{-3}$ there are too few scattered photons to allow for the detection of individual peaks.

Sheffield^{14,15} proposed an elegant solution to this problem by multiplexing the harmonic content of the scattered light spectrum, that is, effectively adding up all the peaks. Physically this entails setting the free spectral range, ω_{FSR} , of a Fabry-Perot interferometer equal to the gyro peak spacing, ω_{ce} . If smearing of the multiplexed harmonics is to be avoided this matching must be achieved with an accuracy better than the

reciprocal of the number of peaks that will be detected, that is, 0.6% in the present experiment.

Inherent in the proposal is the fact that the scattering directions containing modulated light can be identified as 'bright-up' spots in azimuth of the Fabry-Perot fringe, Fig. 4. The scattered spectrum exhibits modulation in only one plane, defined by the scattering geometry and field direction. The intersection of the plane and the Fabry-Perot ring pattern results in a partial fringe, which is measured as an azimuthal angle ' μ '. The Fabry-Perot has advantages such as high throughput and retention of the angular information through the disposition of the partial fringes.

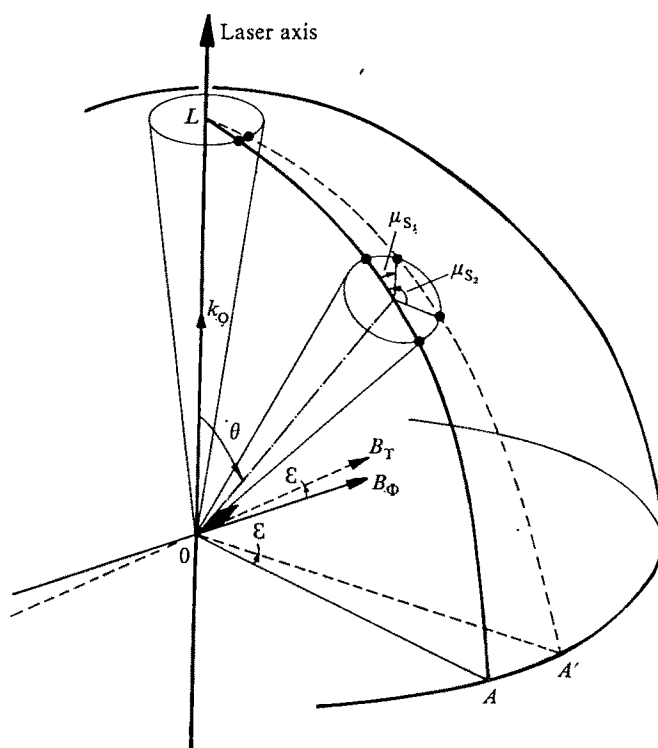
The interpretation of the azimuthal fringe shift ' μ ' in terms of magnetic field directions is given in a generalised treatment by Carolan¹⁶ which holds for all scattering arrangements and plasma field configurations. An illustration of this, suitable for tokamak application, is shown in vector form in Fig. 3. When the scattered light is scanned along the laser beam, the wave vector plane containing the modulated spectrum, rotates about the laser beam direction due to the B_θ variation (see also Fig. 1b). Two examples are shown, with and without a B_θ field component; the cones represent collected scattered light directions. In the case where $B_\theta = 0$ the 'bright-up' of the fringes occur in the plane LOA; when B_θ is finite they appear in LOA'. For larger values of the scattering angle θ the plane of modulated light gives larger values of azimuthal angles μ_{s1} and μ_{s2} , for the same B_θ field. As azimuthal information is preserved unaltered in the detection optics, these azimuthal angles will be represented in the partial interference fringes of the Fabry-Perot interferometer (see Fig. 4).

Constraints on the experiment

Various physical effects can broaden the width of the individual spectral peaks. A complete description of the scattering process for electrons in a magnetic field, which includes all the relevant

Fig. 3 Optical magnification of the inclination angle of field lines. The modulated spectrum in the plane perpendicular to the magnetic axis is contained in LOA. For a field line tilted by angle ϵ the modulation is in plane LOA'. The intersection of these planes and the collection aperture is equivalent to the locus of the partial Fabry-Perot fringes. It can be seen how the sensitivity increases with the larger scattering angle.

$$\mathbf{B}_T = \mathbf{B}_\theta + \mathbf{B}_\phi.$$



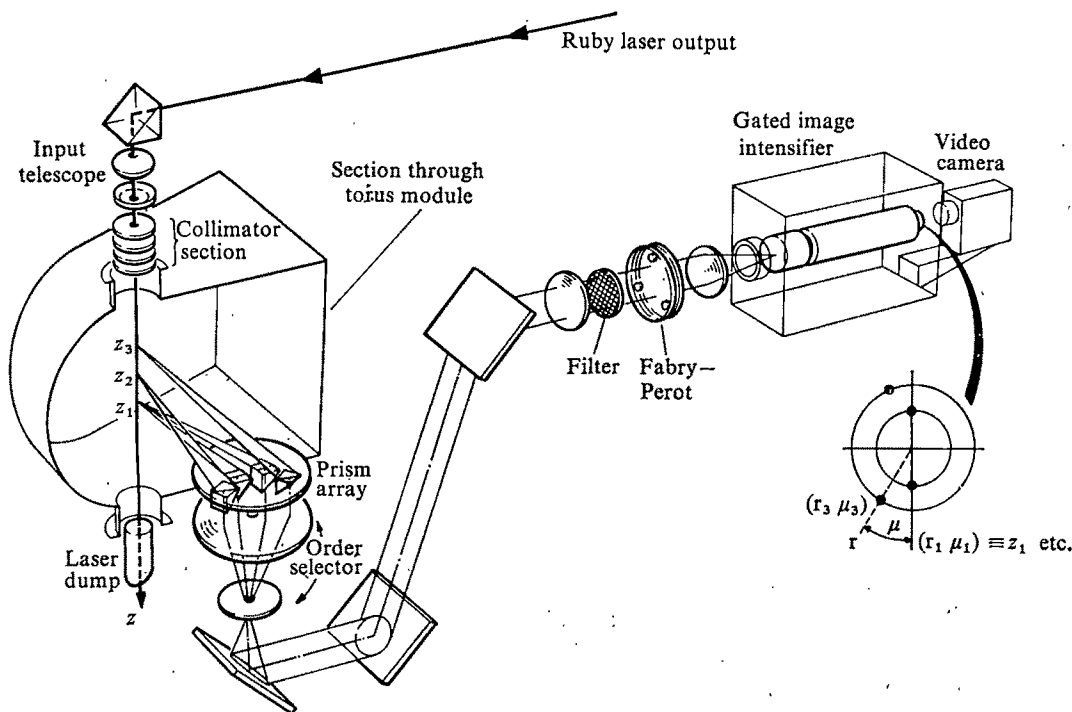


Fig. 4 Schematic of the experimental arrangement on the DITE tokamak module. The four prisms that collect scattered light from selected positions along the laser beam are shown. Variations in B at different positions z_1 , z_2 and so on along plasma radius correspond to different 'bright-up' positions (r_1, μ_1) , (r_2, μ_2) on the Fabry-Perot fringe pattern.

parameters is not available. As the success of the present experiment rests on the simple theory outlined above, it is sufficient for our purposes to show that the conditions chosen were within its validity range. The relevant constraints are listed below with the experimental values in parenthesis.

(1) The bandwidth of the instrumental profile, obtained from the convolution of the laser line profile and the Fabry-Perot transmission profile, must be much less than the peak spacing

$$\omega_{ce} \approx 3.5 \times 10^{11} \text{ rad s}^{-1}, \Delta\omega_{\text{laser-F-P}} \approx 4 \times 10^{10} \text{ rad s}^{-1}$$

(2) The broadening of the spectral peaks introduced by the range of directions of the magnetic field and incident light must be much less than ω_{ce} . Investigations of this problem^{10,17,18} have shown that if the range of k and B is such that $2\nu_e k \cdot B \lesssim B\omega_{ce}$, the peak broadening is small compared to ω_{ce} . This has been used¹⁶ in calculating the limits imposed on the incident laser beam divergence

$$\beta \lesssim \frac{\omega_{ce}}{2k_0\nu_e}, \left[\frac{\omega_{ce}}{2k_0\nu_e} = 1.7 \text{ mrad}, \beta = 2 \text{ mrad} \right]$$

and the range of field variations in the scattering volume

$$\left| \Delta B_0 - \Delta B_\phi \frac{B_0}{B_\phi} \right| \lesssim \frac{\omega_{ce} B_\phi}{k_0 \nu_e \sin \theta},$$

$$\left[\left| \Delta B_0 - \Delta B_\phi \frac{B_0}{B_\phi} \right| = 5 \text{ mT}, \frac{\omega_{ce} B_\phi}{k_0 \nu_e \sin \theta} = 14 \text{ mT} \right]$$

(3) The electrons must execute a number of complete orbits ($\gtrsim 10$) within the scattering volume; this requires

$$\frac{2\pi r_L}{d_s} \ll 1, \left[\frac{2\pi r_L}{d_s} = 0.04 \right]$$

(d_s = scattering volume thickness)

(4) The dispersed light from the Fabry-Perot etalon must have sufficient intensity and contrast to differentiate experi-

mentally between modulated and unmodulated spectra. This requires the utilisation of as many of the spectral peaks as possible due to the small number of photons per peak. A factor limiting the number of useful peaks available is the range of field intensities¹⁶ within the scattering volume

$$\frac{\Delta B}{B_0} \lesssim \frac{1}{kr_L}, \left[\frac{\Delta B}{B_0} = 2 \times 10^{-3}, \frac{1}{kr_L} = 7 \times 10^{-3} \right]^2$$

When this condition is satisfied the light will be modulated to frequency shifts of at least $k\nu_e$, thus at least 80% of the total intensity of the scattered spectra is deeply modulated.

(5) Long range coulomb collisions suffered by an electron gyrating about a force line can perturb its orbit to such a degree that smearing of the scattered spectrum occurs. The role of collisions has been investigated by several authors¹⁹⁻²¹. According to theory, the effect of collisions becomes serious when the random phase error introduced into the scattered light in one gyro period, becomes a significant fraction of 2π . Because the total phase change experienced by light scattered by a gyrating electron in a gyro-period is typically $kr_L \times 2\pi$, the cumulative effect of many long range coulomb encounters becomes important long before a 90° deflection of the electron has occurred (that is a 'collision'). The various theories can be summarised by stating that the scattered spectrum will be modulated, provided $(kr_L)^2 \nu_e / \omega_{ce} \lesssim \alpha$. For 75% modulation, calculations based on, say, the theory of Farley¹⁹ gives $\alpha = 0.05$, while Peratt's²⁷ calculations suggest $\alpha = 2$ (ν_e is the Spitzer²² electron collisional frequency). Experimental evidence¹¹⁻¹³ shows that the role of collisions has been somewhat overestimated, which suggests values of

$$\alpha > 2, [(kr_L)^2 \nu_e / \omega_{ce} \approx 1 \times 10^{-2}].$$

Scattering experiment on a tokamak

The DITE tokamak²³ was operated in plasma conditions which gave the optimum parameters for detecting the modulated spectrum. Values of electron temperature, plasma density and toroidal field were $T_e \approx 350 \text{ eV}$, $n_e \approx 2 \times 10^{19} \text{ m}^{-3}$, $B = 2 \text{ T}$. The observation time was set at 70 ms from current zero, when there was a low level of X-ray activity and electrical

diagnostics indicated a stable plasma. At this relatively low temperature the number of gyro peaks that need to be superposed is reduced. A nominal scattering angle of 30° was chosen as the best compromise between experimental accuracy and the various smearing effects.

The experimental arrangement is shown in Fig. 4; it includes novel selection optics that utilise a resonant Fabry-Perot-prism combination to provide spatial resolution and high optical efficiency. The laser, interferometer and fringe detection system are mounted on a very stable trolley and the laser input and collection optics and the laser dump are attached to the torus module. The output of an Apollo Q-switched ruby laser (output 10 J in 15 ns) is deflected into the vertical axis by a prism and reduced by an inverted telescope to a parallel 1 cm diameter beam (divergence 1.2 mrad) which passes through the magnetic axis of the tokamak. Stringent measures are taken to keep the stray light level low—by collimating light absorbing baffles on the input and an efficient laser dump. This was necessary, as the detection system was designed to have maximum throughput and is consequently of low contrast. Scattered light is collected through a re-entrant window 75 mm diameter, which is level with the torus wall but 8.5 cm behind the plasma limiter. Situated directly behind the window is an array of four anti-reflection coated prisms, cut so that each takes light from a known scattering volume and the set as a whole produces near paraxial rays on their output faces. A convex lens projects these rays onto a focal plane top. Figure 4 shows how the rays from the outer prisms are more convergent than those from the centre prisms. This difference in angles, with a 15 times expanding telescope, is used to force the light into two predetermined interference fringe orders. Each half of each order then has a spatial correspondence with the scattering volume selected by the prisms. The spatial resolution, ≈ 5 mm, is determined by the back projection of the partial fringe into the scattering column.

The inclination of the field lines varies with radial position. Figure 3 shows how the plane containing the modulated spectra moves across the collection port. It is, therefore, important to position the collection prisms to intercept this plane. An estimate of the position of these planes is made from a nominal B_θ distribution and relies on the relatively large prism ($\pm 15^\circ$) to cover any deviation from this estimated position. Crossed wedge prisms are used to compensate for the displacement of the collection prisms, to ensure the light passes through the focal plane tops.

A 15 nm interference filter is used to isolate the scattered

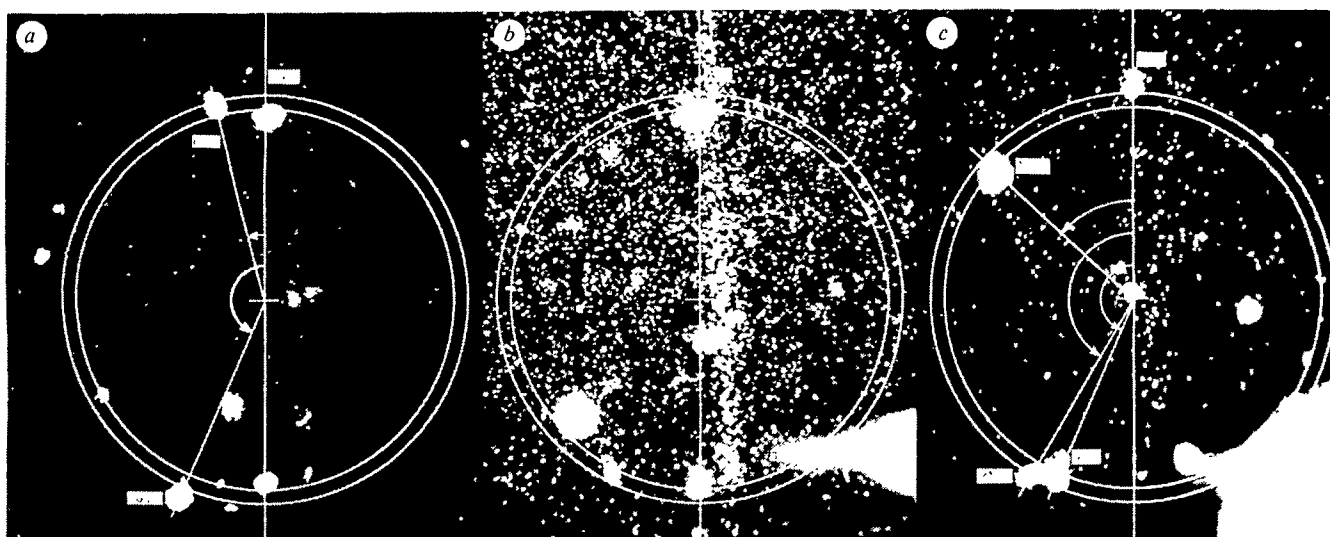
light before it is matched into the Fabry-Perot. The latter has an effective aperture of 13.5 cm and is dielectrically coated to give 70% reflectivity at 694.3 nm, a finesse of 9, that is, designed for high transmission rather than contrast. The etalon spacing is set to 2.72 mm by optically contacted pads; this matches the free spectral range to the gyro frequency for a 2 T field. (For high stray laser light conditions a high reflectivity coating can be used and the etalon spacers matched to $2\omega_{ce}$ to tune out the 694.3 nm light.) A convex lens focuses the fringe system on the extended red photocathode of a gated (50 ns) image tube RCA 73435 AK. This is fibre optic coupled to a three stage intensifier RCA 8606 which gives a luminous gain of 3×10^5 . A video camera, an EMI intensified Ebitron, is used to record the intensified partial fringes on magnetic tape. The recorded pattern is displayed on a TV monitor for processing. An earlier experiment by Forrest, Muroaka and Peacock²⁴ estimated a basic system sensitivity of 18 photoelectrons per degree of azimuth; the number of scattered photoelectrons here is expected to be 60 to 150 per degree of azimuth.

The first pre-aligned prism array is designed to accept scattered light from the magnetic axis, 6 and 10 cm above the axis. The toroidal field was varied in increments until partial fringes were first observed, indicating the matching condition was satisfied. Three distinct sets of fringes, for example, Fig. 5a, were obtained, while on other shots correlation was necessary to distinguish the 'bright-ups' from noise spots; the stray light was not detectable. To ensure the 'bright-up' spots coincided with the selected Fabry-Perot orders, reference fringes were induced by tapping some light off the laser beam, in lieu of stray laser light. The quality of these was poor due to exposure problems, so they have been drawn in for clarity. As a check the plasma current was reversed; this tilts the field lines and hence the plane containing the modulated light in the opposite direction. In this case, partial fringes were only present from the two prisms looking at the magnetic axis, Fig. 5b. This is as expected, because no prisms were positioned to intercept the tilted planes. As a further test the tokamak was run with both the plasma current and toroidal field reversed, and again the four 'bright-ups' were observed.

To complete a radial scan a second prism array was used; here the prisms were set to look on the magnetic axis and at 4 cm, 15 cm and 21 cm above the axis. Partial fringes were again observed but this time with a different azimuthal, Fig. 5c.

From the video data the azimuthal shifts were related to field inclination angles and as B_θ is known, B_z for each

Fig. 5 Video output data showing intensified partial Fabry-Perot fringes labelled with their spatial correspondence. The circular reference fringes are drawn in for clarity. a, First prism array; b, first prism array with plasma current reversed; c, second prism array, plasma current and toroidal field reversed. The background noise on (a) and (c) has been suppressed by reducing the brightness of the video system.



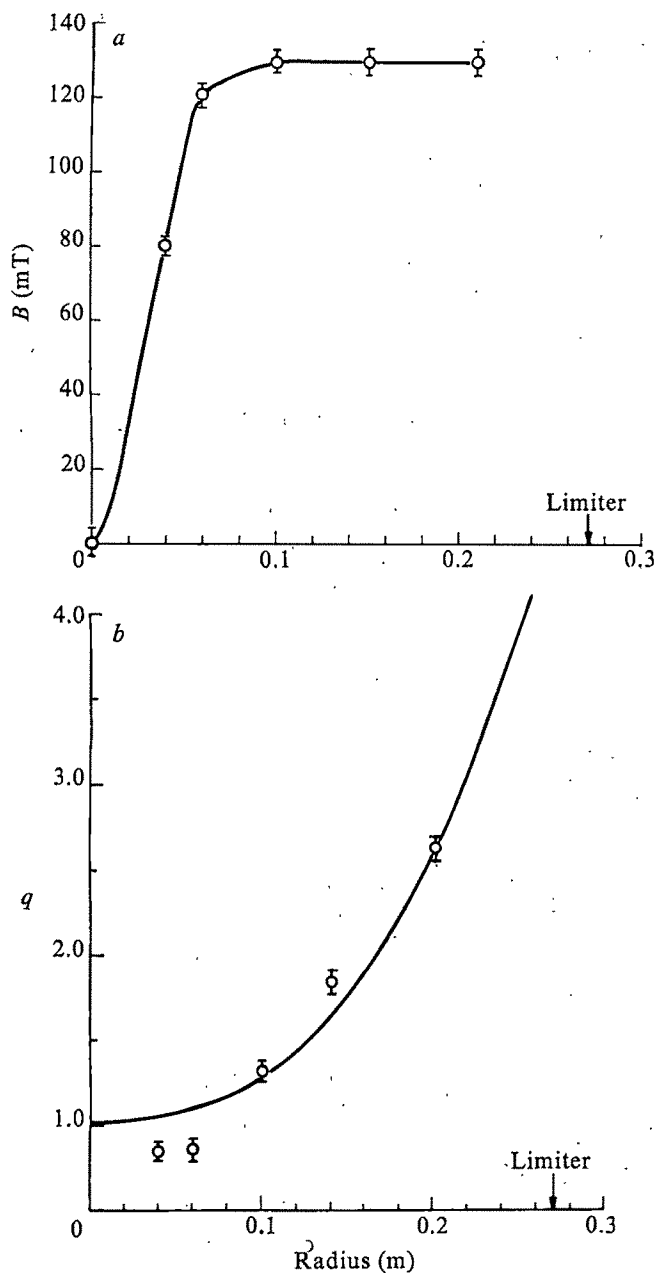


Fig. 6a The measured poloidal field $B_0(r)$ distribution. b, Comparison of 'q' distributions, \circ from poloidal field measurements and — electron temperature profiles assuming $j \propto T_e^{3/2}$ and constant Z_{eff} in similar plasma conditions.

selected radial position is calculated, Fig. 6a. This profile represents a gas current I of 135 kA, while the measured current was 130 kA.

From this poloidal field distribution, $B_0(r)$, we derive a 'q' profile. This is compared with $q(r)$ calculated from the electron temperature profile obtained by previous scattering measurements under similar plasma conditions, Fig. 6b, where it is assumed the current density $j \propto T_e^{3/2}$ so that

$$q(r) = \frac{2\pi r^2 B_0 \int_0^a T_e^{3/2} r dr}{\mu_0 R I \int_0^a T_e^{3/2} r dr}$$

Accuracy of technique

The purpose of the diagnostic is to measure the magnetic field direction in the toroidal coordinates of the tokamak device.

The field direction is measured in the scattering geometry coordinates and then from the orientation of these with respect to the machine, the magnetic field vector in toroidal coordinates can be calculated. Accuracy in determining the former depends on the sensitivity of the directions of modulated scattered light on the field directions: the greater the scattering angle, the more sensitive the dependency¹⁶. An estimate of the accuracy by which the field direction can be measured is obtained from the product of the sensitivity and the range of scattering directions where the light is modulated. For $\theta = 30^\circ$, $T_e = 350$ eV, the ratio B_θ/B_ϕ can be measured with an accuracy $\Delta(B_\theta/B_\phi) = 0.3\%$. The problem of orientating the laser beam and detection optics to the torus was alleviated by the precision machining of the diagnostic port faces. Before fitting the prism array to the torus, it was set up on a test rig, and back projection of an expanded helium-neon laser beam indicated a spatial accuracy of ± 2 mm in centring the selected scattering volumes. Our measurements depended on the location of the azimuthal position of the partial fringes on a video system. Examination of these basic data, Fig. 5, shows that this is somewhat subjective, but it seems possible to determine the angular positions μ to within 0.4° . This is equivalent to determining the field line inclination to within 0.15° or a $\Delta q < 5\%$, which is close to the basic theoretical accuracy of the method.

Conclusions

Magnetic field profiles can be measured by light scattering from the free electrons in tokamaks causing no perturbation to the plasma medium. Basically the method involves detection of the modulation in the scattered light spectrum due to cycloidal motion of the electrons around the field lines.

The sensitivity of the modulation to the perpendicularity condition ($\mathbf{k} \perp \mathbf{B}_T$) ensures a high degree of accuracy in determining the pitch of the magnetic field line—to within 0.15° .

We have demonstrated a detection system that magnifies small changes in the pitch angle of magnetic field lines. The sensitivity has been optimised by using as large a scattering angle as is consistent with conditions for modulation to be satisfied. It has also been shown that the criteria required to observe modulation, in the present experiments, lie within the validity of simple theory.

An extension of the technique to future tokamaks seems promising.

We thank D. E. Evans, J. Sheffield and J. Katzenstein for their support. The successful application of the technique to tokamaks is due to help from J. Hugill and J. W. M. Paul and the DITE diagnostic and operations group.

Received 26 October; accepted 14 December 1977.

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ARAKS—Controlled or puzzling experiment?

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The injection of an electron beam into the magnetosphere has been considered a straightforward technique for studying the large-scale structure of the Earth's environment. The large current released in the magnetosphere by the ARAKS experiment has produced many results which are not yet well understood.

ARAKS (Artificial Radiation and Aurora between Kerguelen and Soviet Union) the Franco-Soviet project, was designed to study the injection of an electron beam into the ionosphere and magnetosphere. The first phase was the launch of two Eridan rockets from the Kerguelen Islands (70° 22' W, 49° 35' S) on 26 January and 15 February, 1975. The final stage of each rocket included two complementary experiment systems: an electron gun, indirect potential measuring devices, particle flux detectors, and a cone ejected at a speed of 40 m s⁻¹ from the main payload. This cone carried antennas to detect radio waves generated by the electron beam and its subsequent interaction with the ionosphere. Great efforts were made to have a truly controlled experiment: it was possible to change the energy and intensity of the electron beam, to vary the pitch angle during injection and the relative trajectories of the nose cone and the main payload were accurately determined. This could be accomplished as previous experiments had already yielded extensive results¹⁻³. Many ground-based measurement facilities were set up in conjunction with this experiment, with emphasis on optical and radar measurements in the Northern hemisphere, at the magnetically conjugate point of the Kerguelen Islands as well as on the VLF and VHF measurements at both points.

In addition, Arcas rockets placed parachute deployed X-ray detectors at ~ 80 km above Kerguelen just before each Eridan launch, to verify that there was no large geomagnetic disturbance under way, and again while the electron gun was operating. The X-ray experiment was performed by the University of Houston.

Objectives

The study of the injection of energetic electrons in the upper atmosphere can be divided into three, each subject requiring a different technique of study, although they are complementary from a physical point of view: first, ionisation and visible phenomena (aurora) at the magnetically conjugate point of the

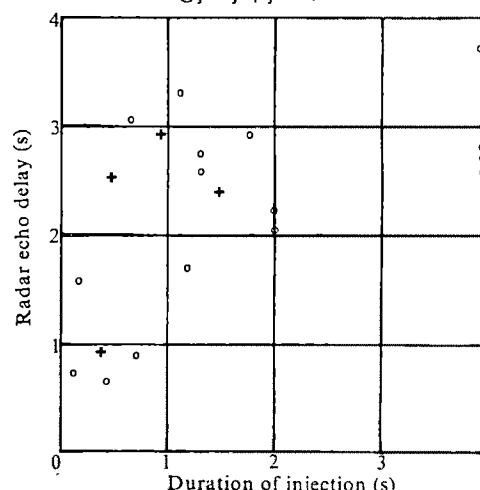
launch site, second, dynamics of injected particles, and third radio waves generated by the electron beam and the effects of wave-particle interactions on the beam itself. A satisfactory compromise was reached between these objectives and the constraints imposed by a space experiment. Two energies were chosen (15 keV and 27 keV) to determine the influence of magnetospheric electrostatic fields on particle trajectories.

Three pitch angles (0°, 70°, 140°) were used to study the following: (1) the production of artificial aurorae and the atmospheric backscattering of the particles at the conjugate point; (2) the magnetic reflection at the mirror point; (3) atmospheric backscattering of the injected electrons in the southern hemisphere.

A variable pulse duration (20ms, 1.28 s, 2.56 s) was adapted in order to have either an accurate definition of the injection angle (20 ms) or a large amount of energy deposited in the atmosphere (2.56 s and a current of 0.5 A).

One of the rockets was fired towards magnetic east in order to compensate for the curvature and gradient drift of reflected electrons in the magnetic field. The other rocket was fired towards the north (26 January), first, in order to reduce the

Fig. 1 Radar echo delay ($\pm \sim 0.01$ s) for different duration of the gun pulse sequences with specific electron pitch angle of injection. \circ , 0°; +, 70°.



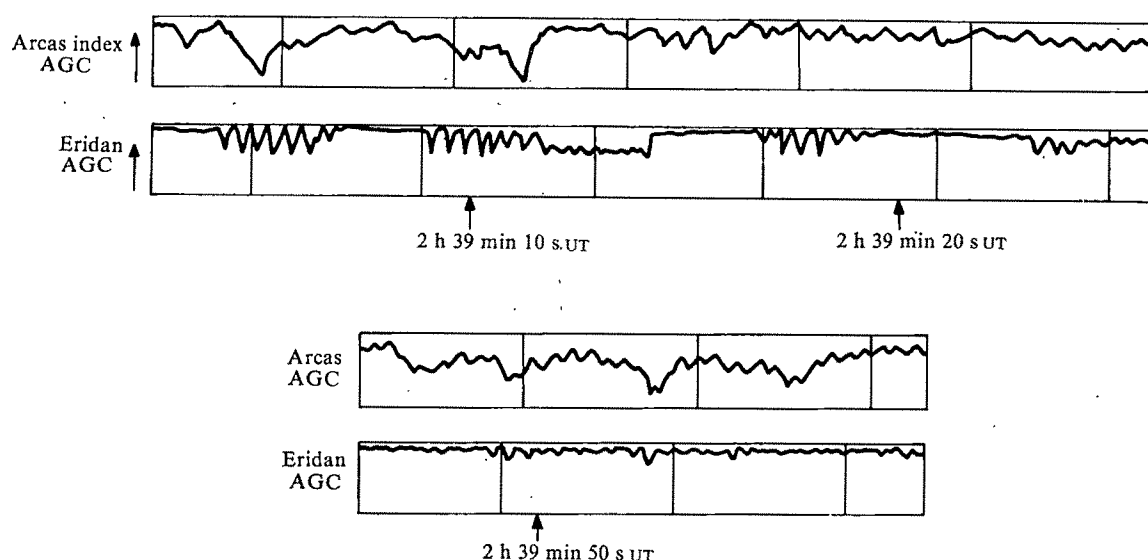


Fig. 2 Telemetry signal strength (AGC) from the X-ray (ARCAS) and ARAKS (Eridan) payloads. In each case the highest signal is best and the lowest is poor, about half way between these two extremes the signal is unsuitable for the recovery of useful data.

size of the impact area of the beam at the conjugate point and thus to facilitate observation of luminous phenomena, and second to obtain more information from the observation of the beam emission, due to a better trajectory of the cone with respect to the main payload.

We shall emphasise here those results which remain problematic, either in comparison with previous experiments or in relation to theoretical ideas prevailing in this field.

Phenomena observed at the conjugate point

Climatic conditions did not permit definitive observations of the optical effects in the atmosphere: the first launch could not take place during the astronomical twilight, and during the second launch there was some cloud cover. It is, therefore, difficult to draw conclusions and we can only say that the brightness of the artificial aurorae in any case was not brighter than that of a mag 7 star (the TV device's sensitivity permitted the observation of stars down to mag 9).

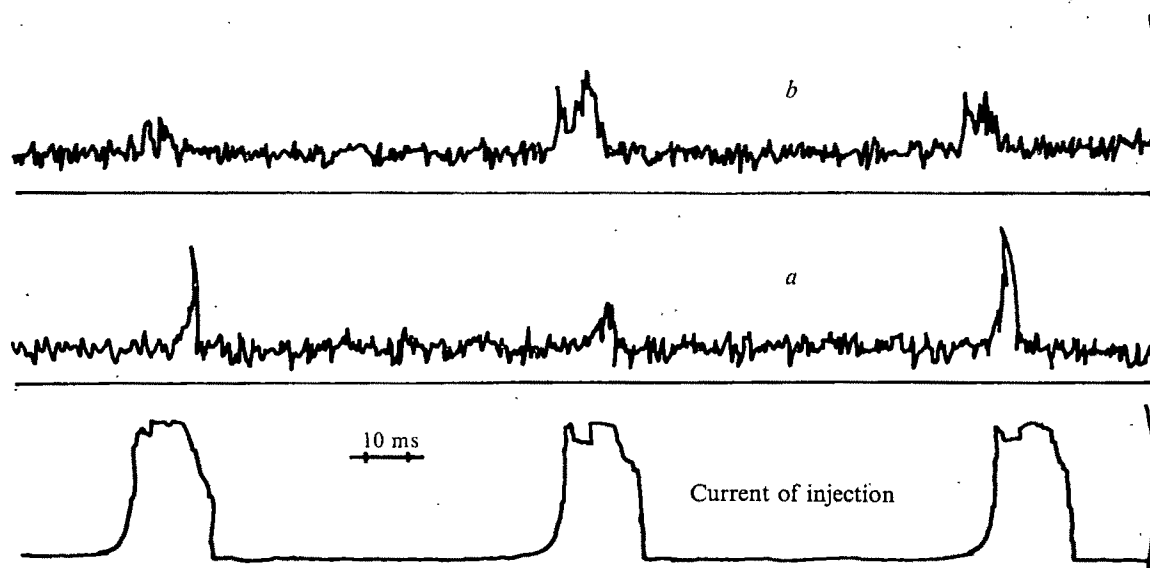
Artificial aurorae were, however, clearly detected in the magnetically conjugate area by means of pulsed and continuous wave radars operating at 23 and 44 MHz, in the Kostroma and Vologda areas (during the whole first flight and during the last

third part of the second flight). The measurement precision is 2 km in the north-south direction and 10 km in the east-west direction. For the first launch the results agree well with predictions made from the POGO (8/71) and GSFC (12/66) geomagnetic field models. For the second launch, the agreement between the measured and theoretical conjugate point is less satisfactory; however, we note that the magnetic activity index was higher during the second launch ($K_1 = 4$ VS, where $K_1 = 1$ for the first launch).

The variation in distance perpendicular to the line of sight of radar echoes is caused by the lateral movement of the rocket during electron injection. The detailed study of radar echoes coming from the impact of electrons in the neutral atmosphere is more important. We detected the presence of two types of radar echoes, which could be distinguished by a variation in the Doppler effect corresponding to a speed variation from 30 to 200 m s⁻¹. The spectra obtained for the low-velocity component are narrower than the equivalent spectra observed in natural radio aurorae.

The time difference between the onsets of the injection of electrons into the ionosphere at Kerguelen and the onsets of the radar echo at the conjugate is surprising. Figure 1 shows the

Fig. 3 Radio noise at Kerguelen: *a*, 50 and *b*, 75 MHz. The lowest curve represents the current of the injected electron beam.



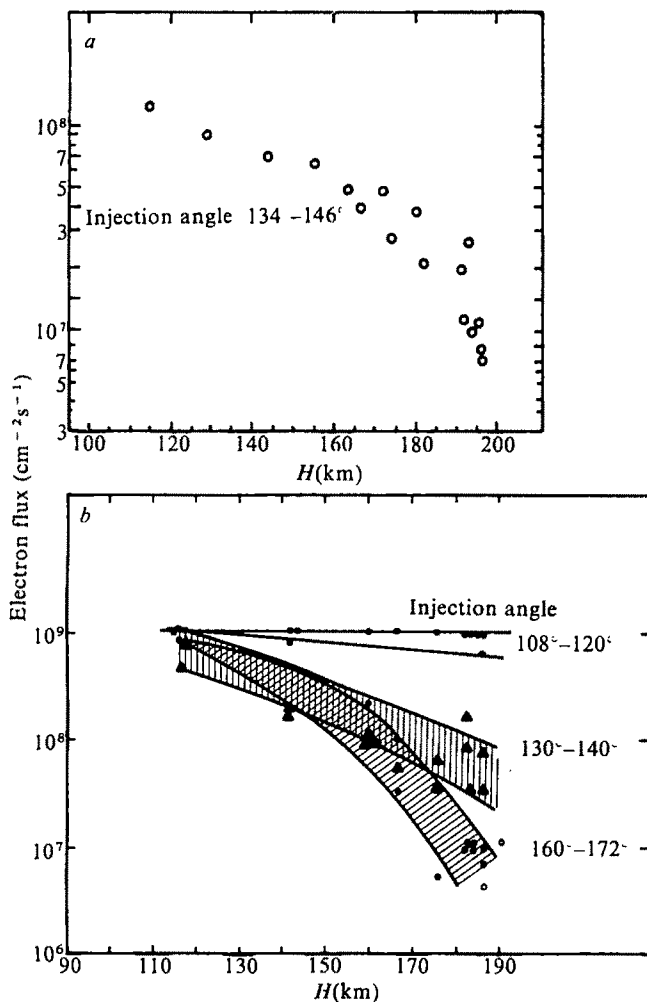


Fig. 4 Electron fluxes ($E > 8$ KeV) observed by the wide angle detectors for downward injections. *a*, 26 January 1975; *b*, 15 February 1975.

scattering of radar echo delays for the first flight for different durations of the gun pulse sequences with specific electrons pitch angle of injection. The calculated time for the transit between conjugate points is 0.65 s for 27 keV electrons, and 0.85 s for 15 keV electrons. Apart from the anomalously high delay times shown in Fig. 1, we also observed that radar echo durations were longer than corresponding gun pulse durations (up to more than 10 s). Both unexpected delays and durations of radar echoes could be due to beam behaviour close to the rocket or beam-plasma interactions along the beam path or ionospheric processes at the conjugate points.

The ejection of electrons without a return current would necessarily carry the main payload, including the gun, to a very high positive potential which would thus eliminate electron emission. When such an injection occurs in a rarefied environment, ionised or not, there exist processes capable of generating these return currents (collection of thermal electrons, ionisation of neutral gas and so on). To facilitate collection of the return currents, two techniques may be used: either to increase the collection surface¹, or to increase the conductivity of the environment by injecting a plasma (Argon plasma was used in the Electron Echo Experiments² and a caesium plasma in ARAKS). This experiment had clearly shown that plasma source causes a large scale disturbance in the payload environment, consequently exerting an influence on gun neutralisation.

The AGC (Automatic Gain Control) of the telemetry signals of the Eridan and Arcas payloads show similar disturbances during the first minute of the active phase of the 26 January ARAKS experiment, although the telemetry frequencies are

quite different (250 MHz and 1.68 GHz) and the two payloads are 80 km apart on the same geomagnetic field line. Figure 2 shows part of these data. A large disturbance occurred in the Eridan signal at about 2 h 39 min 10 s UT when the plasma source was turned on and intensified 1.5 s later when the first long duration gun pulse occurred.

Among the electrons constituting the return current we can distinguish a population on all altitudes having a higher temperature than that of ionospheric plasma (0–300 eV) and another population of energetic electrons (1–3 keV) at higher altitudes (> 120 –130 km). Available data are not sufficient to draw certain conclusions on the rocket potential: ~ 125 V or 1.5–2 kV above 120–130 km; on lower altitudes it is possible to deduce from particle measurements one rocket potential value-definitively $< \sim 100$ V.

The increase of the electron density near the rocket is confirmed by the existence of high frequency radio waves (10–75 MHz) with a wide bandwidth. Figure 3 shows the radio noise data at two frequencies (50 and 75 MHz) detected at Kerguelen when the electron gun was operating. This noise had already been observed in the Zarnitza 1 experiment³; with ARAKS, it was generated in a direction very different from that perpendicular to the magnetic field lines. The measurements should coincide with the presence of a halo around the rocket, visible from the ground when it is at a low altitude. The present results show that the mechanisms of neutralisation, which exist whatever other conditions are involved, are so complex that these observations do not permit a full understanding of them.

Atmospheric electron scattering

Intense electron fluxes ($E > 8$ keV) were observed on the rocket by the wide angle detectors during the gun firings. For downward injection, the measured electron fluxes depend on the pitch angle of injection and on the rocket altitude. The measured fluxes at the same altitude were different for the two flights, perhaps because of large differences in the atmospheric density (Fig. 4). There is a good agreement between the intensities of measured fluxes and the values calculated by a Monte-Carlo method for downward injection. For upward injection, the measured intensities of electron fluxes are several orders of magnitude higher than those calculated by the Monte-Carlo method. Thus it seems that electron scattering by the atmosphere is an important process during downward injection; at present, there is no satisfactory interpretation of the results obtained during upward injections.

Wave particle interactions; wave emission

As expected, the electron beam generates radio waves when penetrating the plasma. Among the various devices used for the

Fig. 5 26 January 1975 H F results. Grey intensity indicates the amplitude of the waves the frequency of which is along the ordinate. The abscissa is the time of the flight and at the bottom the gun sequence is represented.

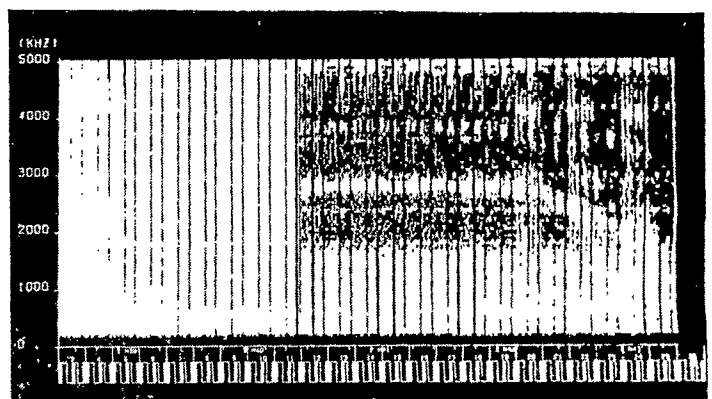


Table 1 Comparison of Electron echo experiments with ARAKS

	Electron echo experiment 1 ($I = 70$ mA)	Electron echo experiment 2 ($I = 70$ mA)	ARAKS ($I = 0.5$ A)
$f_D < f < f_H$	Wave front $V < 3$ mV	Continuous emission	Wave front $V < 200$ μ V
$f \approx nf_b$	Yes $V < 100$ μ V	Yes	$\omega \approx 4\omega_b$ mainly for the first flight
$f < f_b$	Wave front	Continuous emission $V \approx 300$ μ V	Continuous emission even at $V = 500$ μ V
Low frequency	Wide band noise around the LHR (argon source)		Wide band noise with E and B component (electron gun) Monochromatic electrostatic wave (caesium source) $V \approx 100$ μ V

study of waves generated by the beam, we used for the first time a wide bandwidth telemetry system transmitting a signal waveform up to 5 MHz. Thus we could measure the time evolution of wave spectra with high resolution in the frequency/time domain. It was found in the 0.1–1 MHz range that the time structure of the radio impulse repeats exactly that of the gun impulse, while this is not the case for higher frequency waves.

Figure 5 gives a general representation—analogue to a 'sonagram' of the HF part of the received waves. It must be noted that during the north flight the lateral separation of the nose cone from the beam trajectory varies from 200 m up to 1.5 km. Table 1 summarises the observations comparing them to previous ones². For the frequencies located between the plasma frequency and the upper hybrid frequency the emission due to the gun shows mainly a wave front (Fig. 6) probably due to the coherent part of the spontaneous radiation^{4,5}. In the whistler mode, that is, for frequencies lower than the electron gyrofrequency, ARAKS gives an important result: even at a large distance perpendicular to the beam a continuous radiation is observed. This suggests the existence of some mechanisms by

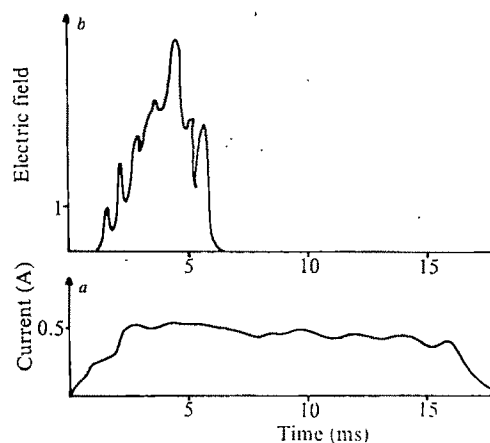


Fig. 6 *a*, Shape of the electron pulse; *b*, corresponding amplitude of the waves generated close to the plasma frequency in arbitrary units.

which part of the unstable modes trapped in the beam are converted into radiated modes.

For very low frequencies, a very large difference appears during ARAKS which until now has been unexplained: the caesium plasma source generates a quasimonochromatic electrostatic wave (no magnetic component can be detected). The frequency of which changes from 4.5 kHz down to 3.5 kHz. The caesium may have reached the ejectable cone where these measurements were being made.

In conclusion, ARAKS has made a remarkable contribution to plasma physics (discharge of a plasma beam generation of instabilities in an unlimited environment) as well as in geophysics (topography of the geomagnetic fields, analogy with VLF hiss). Some of these points are difficult to explain and we must examine them in detail before claiming that we are really able to perform 'controlled' experiments.

Received 1 August; accepted 9 November 1977.

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Novel cell cycle control of RNA synthesis in yeast

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During the fission yeast cell cycle, the rate of polyadenylated messenger RNA synthesis doubles when the cell reaches a critical size. This size-related control maintains average mRNA content in balance with total cell mass during exponential growth, even in cells growing at different absolute growth rates per cell.

In cell cultures undergoing balanced exponential growth, individual parameters of growth remain at a constant proportion of total cell mass¹. The two daughter cells produced at each division are identical to the parent at the same time in the

preceding cycle: this requires that all cell components are doubled during the course of each cell cycle. The mechanisms controlling the balanced increase of components, and their duplication during each cell cycle are not understood.

A simple model explaining the doubling in amount of a component in each cell cycle can be based on a stepwise doubling in the rate of synthesis of that component at a fixed point in every cell cycle. In the cell cycle of the fission yeast *Schizosaccharomyces pombe* there are periodic doublings in rate of increase of total dry mass², rates of increase in activities of three enzymes³, and in rates of synthesis of total RNA⁴, ribosomal protein⁴, ribosomal RNA⁵ and polyadenylated mRNA⁵. In all cases, the doubling in rate occurs early in the cell cycle.

Two types of control may be proposed to account for the timing of the rate doublings during the cell cycle. The doublings

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Table 1 Characteristics of mutant and wild-type strains

	Wild type 972 h ⁻	Mutant <i>wee</i> 1-50
Generation time (min)	140	140
Cell volume at division (μm^3)	149	73
RNA (pg per cell)	3.0 ± 0.04	1.59 ± 0.07 (53%)*
Protein (pg per cell)	12.1 ± 0.3	6.85 ± 0.4 (57%)
PolyA ⁺ mRNA (fg per cell)	23.8 ± 0.8	14.1 ± 0.4 (59%)
DNA (fg per cell)	34.6 ± 1.4	28.4 ± 0.8 (82%)

Cultures of the two strains were grown at 35 °C in phthalate-buffered EMM 2 medium. The length and diameter of 200 cells with cell plates were measured and cell volume calculated. Cell generation time, RNA, protein and DNA contents were determined using cultures in the early phase of exponential growth by methods described in ref. 7. PolyA⁺mRNA content was calculated from the ultraviolet absorption spectrum of polyA⁺mRNA isolated by affinity chromatography on oligo dT-cellulose as described in ref. 5. Polyacrylamide gel electrophoresis of the polyA⁺mRNA fraction showed no contamination by ribosomal or transfer RNAs. Values are means \pm s.e.m.

*Numbers in parentheses give the values of parameters determined in the mutant as a percentage of that determined in the wild type.

might be dependent on DNA replication, which occurs at the beginning of the fission yeast cell cycle⁶. Alternatively, the rate doublings may be related to overall growth, being triggered when the cell reaches some critical size or mass. These two types of control mechanism may be distinguished using strains of fission yeast which have very different cell sizes⁷ but in which there is relatively little variation in the timing of DNA replication in the cell cycle⁷. We have investigated the rates of synthesis of polyadenylated mRNA (polyA⁺mRNA) during the cell cycles of cells dividing at different sizes. Our evidence suggests that the timing of the doubling in rate of polyA⁺mRNA synthesis in the cell cycle is not controlled by the timing of DNA replication, but that doubling in the rate of mRNA synthesis is triggered when the cells reach a critical size. Such a mechanism will maintain the average ratio of polyA⁺mRNA to total cell mass, and will enable cells of different mean sizes to double their mRNA content in each cell cycle.

Characteristics of the wild-type and mutant strains

Table 1 shows generation times and macromolecular contents of exponentially-growing haploid cells of wild type strain 972 h⁻ and a mutant derivative *wee* 1-50 h⁻. The mutant strain (previously described as *cdc* 9-50 (ref. 7)) is altered in the control initiating mitosis, so that mitosis and cell division take place in cells of about half the size of wild type⁷; generation time is unaltered. Growing cells of strain *wee* 1-50 have a reduced macromolecular content per cell, with 57% of the protein, 53% of the total RNA and 59% of the polyA⁺mRNA content of wild type. The average DNA content per cell of *wee* 1-50 is comparatively high, at 82% of wild type. This results in a ratio of polyA⁺mRNA to DNA which is lower in *wee* 1-50 than in wild type, and therefore mRNA content per cell cannot be regulated by gene dosage alone.

Macromolecular synthesis during the cell cycle

To examine the means by which the reduced polyA⁺mRNA content per cell of *wee* 1-50 is brought about, we measured the patterns of synthesis of DNA and polyA⁺mRNA in synchronously-dividing cultures of *wee* 1-50 and wild type. In wild type cells, DNA replication was at the beginning of the cell cycle, almost coincident with cell division (Fig. 1). The rate of incorporation of adenine into polyA⁺mRNA doubled as a step, with the midpoint of the step about 0.1 to 0.2 of a cycle after the midpoint of DNA accumulation. Similar results have been obtained using ³H-uridine as label instead of ³H-adenine, and also with another strain of wild-type size⁵.

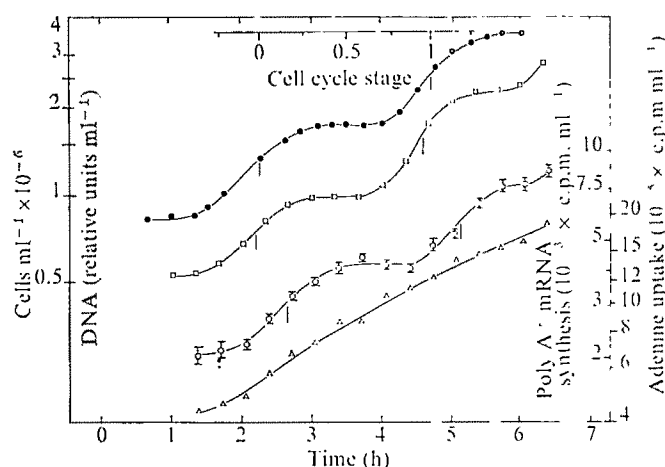


Fig. 1 Changes in cell no., DNA content and rates of adenine uptake and polyA⁺mRNA synthesis in a synchronously-dividing culture of *Schizosaccharomyces pombe* Lindner, wild-type strain 972 h⁻. Cells were collected by filtration from a population growing exponentially at 35 °C in phthalate-buffered EMM2 medium⁷, and were fractionated by sedimentation rate centrifugation through a lactose density gradient¹⁷ (MSE Type A zonal rotor). Small cells at the beginning of the cell cycle were selected as inoculum for the synchronous culture. Changes in cell number were monitored using a Coulter counter. Samples of 50–100 ml culture were withdrawn every 20 min and pulse-labelled by incubating for 10 min (0.07 of the cell generation time) with 2.5 $\mu\text{Ci ml}^{-1}$ [³H]adenine (Radiochemical Centre). The total adenine concentration of the pulse medium was adjusted to 1 $\mu\text{g ml}^{-1}$ by addition of nonradioactive adenine. Less than 10% of the radioactivity supplied was taken up during the pulse. DNA content and rates of adenine uptake and polyA⁺mRNA synthesis were measured as described in ref. 5. Values for polyA⁺mRNA synthesis are means \pm s.e.m. of six determinations. The midpoints of the two successive doublings in cell numbers fix stages 0 and 1 on the cell cycle map. The midpoints of the stepwise doublings in DNA content and rate of polyA⁺mRNA synthesis were taken as the time when the step attained the value midway between the two plateaus on either side of the step, and are marked by vertical bars. ●, Cell no.; □, DNA content; △, rate of adenine uptake; ○, rate of polyA⁺mRNA synthesis.

Quite different results were obtained with synchronous cultures of *wee* 1-50. The midpoint of DNA replication was 0.2 to 0.3 of a cycle after cell division (Fig. 2), consistent with previous results⁷ and with the average DNA content of *wee* 1-50 cells in asynchronous culture being 82% of that of wild type (Table 1). The rate of adenine incorporation into polyA⁺mRNA also doubled as a step, but the midpoint of the step was at an

Fig. 2 Changes in cell no., DNA content and rates of adenine uptake and polyA⁺mRNA synthesis in a synchronously-dividing culture of the mutant strain *wee* 1-50. Measurements were made and the cell cycle stage of changes derived as explained in the legend to Fig. 1. ●, Cell no.; □, DNA content; △, rate of adenine uptake; ○, rate of polyA⁺mRNA synthesis.

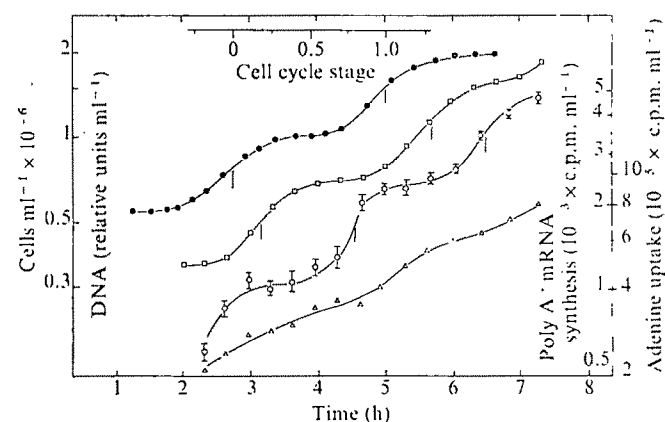


Table 2 PolyA⁺mRNA synthesis during the cell cycles of wild type and *wee 1-50* mutant strains

	Wild type 972 h ⁻¹	Mutant <i>wee 1-50</i>
Cell cycle stage of midpoint of stepwise doubling in rate of polyA ⁺ mRNA synthesis	0.11 ± 0.04	0.81 ± 0.06
Rate of polyA ⁺ mRNA synthesis per cell before stepwise rate-doubling (10 ³ × c.p.m.)	1.24 ± 0.05	1.29 ± 0.03
Rate of polyA ⁺ mRNA synthesis per cell after stepwise rate-doubling (10 ³ × c.p.m.)	2.43 ± 0.15	2.39 ± 0.14

Data were derived from synchronous culture experiments such as shown in Figs 1 and 2. The time of the midpoint of stepwise doubling in rate of polyA⁺mRNA synthesis was expressed as stage in the cell cycle as shown in Fig. 1. Rates of polyA⁺mRNA synthesis per cell before and after the stepwise doubling in rate were calculated by dividing the steady rates of incorporation before and after the step by the cell number per ml after separation of the daughter cells formed at the beginning of that cell cycle. Values are means ± s.e.m.

average of 0.81 of the cell cycle (Table 2). This is 0.5 of a cycle later than DNA replication in *wee 1-50*, and 0.7 of a cycle later than the corresponding step in rate of incorporation into polyA⁺mRNA in wild type. These results suggest that the timing of the doubling in rate of incorporation into polyA⁺mRNA is not directly controlled by the timing of DNA replication.

The pattern of increase in the rate of adenine uptake during growth of synchronous cultures is rather variable (Figs 1 and 2, and ref. 5). However, the stepwise increases in the rate of adenine incorporation into polyA⁺mRNA were greater than any periodic changes in adenine uptake. This suggests that the changes in rate of adenine incorporation into polyA⁺mRNA resulted from changes in rate of synthesis and not from changes in rate of precursor uptake. Control experiments were carried out to check whether the doublings in rate of polyA⁺mRNA synthesis were merely periodic fluctuations induced by the physiological trauma of synchronous culture preparation. Cells from the parent culture were exposed to all the conditions used to prepare synchronous cultures, but the inoculum selected contained cells at all stages of the cell cycle. The resulting culture showed an exponential, asynchronous increase in cell number. The rate of adenine incorporation into polyA⁺mRNA also increased continuously with time: there was no suggestion of the steps in rate of incorporation seen in synchronous culture. We conclude that the stepwise doublings in rate of polyA⁺mRNA synthesis observed in synchronous cultures were cell cycle-related events.

Dependency on DNA replication

Although in the cell cycle of *wee 1-50* the timing of the doubling in rate of synthesis of polyA⁺mRNA is not closely linked with the timing of DNA replication, the doubling in rate of polyA⁺mRNA synthesis might still depend on completion of the previous round of DNA replication. We examined this possibility by following the rate of synthesis of polyA⁺mRNA in an asynchronous, exponentially-growing culture of *wee 1-50* after inhibition of DNA synthesis by hydroxyurea. The rate of DNA synthesis fell to about one quarter of its initial level within 5 min of addition of the inhibitor (Fig. 3). The rate of synthesis of polyA⁺mRNA continued to increase at approximately the same rate as in the control for about 70 min, then the rate became constant. This 70-min delay is similar to the period between DNA replication and doubling in rate of polyA⁺mRNA synthesis in synchronously-dividing cultures of *wee 1-50* (Fig. 2). These results fit the following model: the doubling in rate of synthesis of polyA⁺mRNA is dependent on the occurrence of

the preceding round of DNA replication. After DNA replication, a cell is potentially capable of doubling its rate of polyA⁺mRNA synthesis, but in *wee 1-50* does not do so until 0.5 of a cycle later (Fig. 2, Table 2). In an asynchronous population, there will be a fraction of cells which have completed DNA replication but which have yet to double their rate of polyA⁺mRNA synthesis. These cells are responsible for the 70-min period during which the rate of polyA⁺mRNA synthesis rose after inhibition of DNA synthesis (Fig. 3). Consistent with this interpretation, in cells of wild type size, where doubling in rate of polyA⁺mRNA synthesis follows closely on DNA replication (Fig. 1, Table 2), hydroxyurea added to an asynchronous culture causes the rate of polyA⁺mRNA synthesis to become constant within 10 to 20 min (ref. 5).

Hydroxyurea is the only inhibitor of yeast DNA synthesis known to give the very rapid inhibition required for these experiments⁸. Its use is open to the criticism that hydroxyurea reportedly inhibits precursor uptake and growth (ref. 8 and J. M. Mitchison and J. Creanor, unpublished). Adenine uptake was not inhibited in our experiments (Fig. 3), but we cannot entirely exclude the possibility that hydroxyurea was having some unknown side effect which prevented further rise in the rate of polyA⁺mRNA synthesis.

Explanation for the reduced polyA⁺mRNA content of *wee 1-50*

We have shown that the rate of polyA⁺mRNA synthesis doubles much later in the cell cycle of *wee 1-50* than in wild type. This difference can be shown to be sufficient to account for the

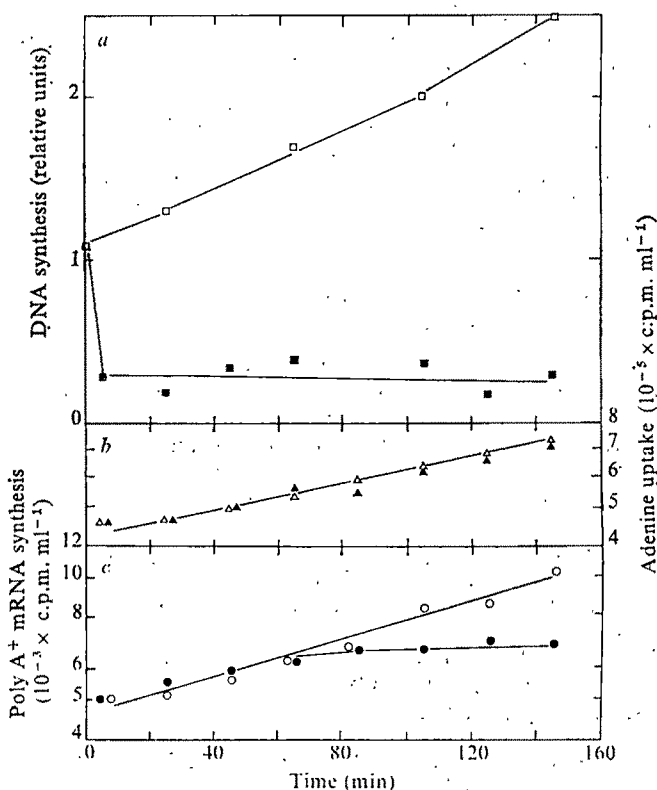


Fig. 3 Changes in rates of DNA synthesis, adenine uptake and polyA⁺mRNA synthesis in a culture of *wee 1-50* after inhibition of DNA synthesis. An exponentially-growing culture containing 10⁶ cells ml⁻¹ was split in two. Hydroxyurea (11 mM) was added to one half at 0 min to inhibit DNA synthesis. The other half served as control. The rate of DNA synthesis was measured as described in ref. 18. Rates of adenine uptake and polyA⁺mRNA synthesis were measured as described in the legend to Fig. 1. *a*, DNA synthesis; *b*, rate of adenine uptake; *c*, rate of polyA⁺mRNA synthesis. Open symbols represent values for the control culture, closed symbols for the hydroxyurea-treated culture.

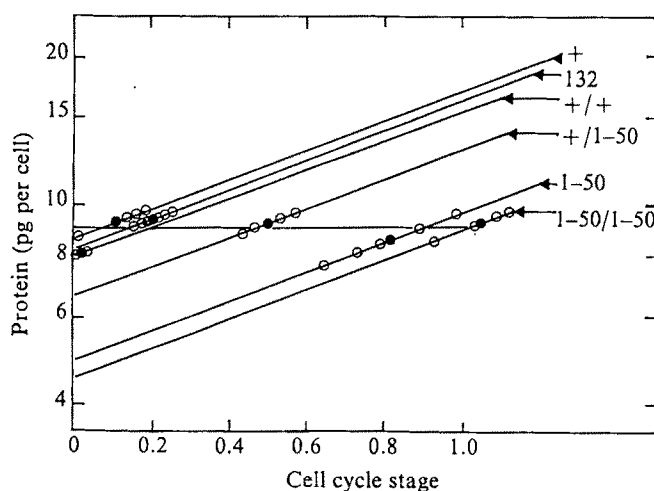


Fig. 4 Relationship between cell protein content and cell-cycle time of the stepwise doubling in rate of polyA⁺mRNA synthesis, in yeast strains of a wide range of sizes. Each open circle represents the midpoint of a stepwise doubling in rate of polyA⁺mRNA synthesis, measured in synchronous cultures as shown in Fig. 1. Closed circles represent the mean values for each strain. The yeast strains used were 972 wild-type haploid (+); strain 132 haploid (132); 972 diploid wild type (+/+); *wee* 1-50 haploid (1-50); *wee* 1-50 diploid (1-50/1-50) and the heterozygous diploid of *wee* 1-50 and 972 wild type (+/1-50). The cell-cycle time scale is the time between the midpoints of two successive divisions. The points for time of doubling in rate of polyA⁺mRNA synthesis are plotted along a line showing the increase in protein content during the cell cycle for each strain. These lines were calculated from the mean protein content per cell in asynchronous, exponential culture for each of the strains knowing that protein content per cell increases close to exponentially during the cell cycle⁹. The protein per cell data for the three diploid lines have been divided by two to permit comparison with the haploid lines.

reduced polyA⁺mRNA content of the average *wee* 1-50 cell in exponential culture (Table 1), by calculating the polyA⁺mRNA content of *wee* 1-50 if the delay in doubling were the only difference between the two strains. The absolute rates of polyA⁺mRNA synthesis per cell before and after the step doubling in rate are the same for *wee* 1-50 and wild type (Table 2). We assume that the half life of the polyA⁺mRNA is the same in both strains. In these conditions, the mean polyA⁺mRNA content of *wee* 1-50 cells in asynchronous, exponential growth is 2^{-l} of the wild-type content, where l is the delay, as a fraction of the cell cycle, between doubling in rate of polyA⁺mRNA synthesis in the two strains. The derivation of this relationship is to be described in detail elsewhere (A. Barnes, P.N. and R.S.S.F., in preparation). The average delay between doubling of the rate of polyA⁺mRNA synthesis in wild type and *wee* 1-50 was 0.70 of the cell cycle (Table 2). This gives a calculated value for mean polyA⁺mRNA content of *wee* 1-50 cells of 62% of the wild-type content. This is in good agreement with the observed value of 59% (Table 1), and indicates that the delay in doubling the rate of polyA⁺mRNA synthesis in *wee* 1-50 cells is sufficient to explain their reduced polyA⁺mRNA content.

Cell size control over polyA⁺mRNA synthesis

We now consider the nature of the mechanism which determines that the rate of polyA⁺mRNA synthesis doubles early in the cell cycle of wild type and late in the cycle of *wee* 1-50. *Wee* 1-50 cells at the end of their cycle are similar in size to wild-type cells at the beginning of their cycle, suggesting that some aspect of cell size might be important. We have studied this quantitatively by taking cell protein content as a measure of size. Knowing the protein content per cell in asynchronous, exponential culture (Table 1), and that protein per cell increases close to exponentially through the cell cycle⁹, we can calculate changes in protein per cell during the cell cycle of each strain. Figure 4 shows that in *wee* 1-50 and wild type, the doubling in rate of

polyA⁺mRNA synthesis occurred in cells with similar protein contents. To examine this further, we have measured the time when the rate of polyA⁺mRNA synthesis doubles in another haploid strain (132) of wild-type size⁵, and in a series of diploid strains of different sizes. All strains investigated showed a stepwise doubling in the rate of polyA⁺mRNA synthesis in synchronous culture; all have similar mean generation times. The protein contents of the diploid strains have been divided by two before plotting (Fig. 4), to allow comparison directly with the haploid strains.

In the six strains, the times at which the rate of polyA⁺mRNA synthesis doubles are distributed throughout the cell cycle. However, all strains double their rates of polyA⁺mRNA synthesis when the cells have very similar levels of protein per haploid genome (Fig. 4). This suggests a cell size-related control over the timing of the doubling in rate of polyA⁺mRNA synthesis in the cell cycle.

We propose that in cells in steady-state growth, the rate of synthesis of the majority of polyadenylated mRNAs is under a general control. This sets the rate per cell at one of two levels, the higher rate being double the lower. The cell switches from the lower to the higher rate on attaining a critical size or mass. The control consists of two parts: the recognition of the threshold size, and the means by which the rate of polyA⁺mRNA synthesis is maintained at the two discrete levels.

Recognition of the threshold cell size might involve monitoring some aspect of cell growth. Such size-monitoring mechanisms have been proposed as controls of initiation of DNA replication and mitosis¹⁰. An example would be the 'inhibitor dilution' model as proposed by Pritchard¹¹. A regulator of mRNA synthesis would be synthesised as a pulse once per cycle. When the concentration of this regulator is reduced to a threshold value by increase in cell volume, the doubling in rate of polyA⁺mRNA synthesis would be effected. Our comparison of haploid and diploid strains suggests that the amount of regulator synthesised would be a constant amount per haploid genome, not a constant amount per cell. We do not know the nature of this regulator, but a promising candidate would be a molecule analogous to 'magic spot' which is involved in a stringent type of control¹².

The simplest mechanism whereby the rate of mRNA synthesis could be maintained at one rate or twice that rate is one based on a modification of the gene dosage model. This type of model is supported by our experiments on the rate of polyA⁺mRNA synthesis after inhibition of DNA accumulation by hydroxyurea (Fig. 3), and by the observation that the absolute rates of polyA⁺mRNA synthesis per cell before and after the step doubling are the same in *wee* 1-50 and wild type (Table 2). However, since the doubling in rate of polyA⁺mRNA synthesis may occur much later than DNA replication, as in *wee* 1-50, there must be some further mechanism to maintain the rate of polyA⁺mRNA synthesis at the pre-replication rate until the threshold cell size is reached. This could involve some form of temporary masking of one of the two copies of each gene present after replication^{3,13-15}, only one of which would be active until the cell had attained the threshold size. Alternatively, there could be some form of gene dosage compensation such as is found in *Drosophila*¹⁶. In this model the rate of transcription of each of the two gene copies present after DNA replication would be half that of the single copy present before replication. The rate of transcription of both copies would be doubled when the cell attained the threshold size. This control could act through the availability of RNA polymerase or its regulatory subunits, though in these cases it is not clear why the rate of transcription should necessarily double as a step.

The proposed general control over rate of mRNA synthesis does not exclude the possibility that the rate of transcription of individual genes is also subject to other, more specific controls.

Significance of cell size control

The cell size-related control over time of doubling in rate of polyA⁺mRNA synthesis has several implications for the control

of balanced exponential growth. It will maintain the average polyA⁺mRNA content per cell as a constant proportion of total cell mass during exponential growth, and will ensure that polyA⁺mRNA content per cell doubles over the course of each cell cycle. This regulation of balanced growth and duplication will operate even in cells dividing at different sizes and growing at different absolute growth rates per cell. Thus the control could maintain the concentration of polyA⁺mRNA within a range suitable for further post-transcriptional modulation.

It has been shown⁶ that a doubling in the rate of mRNA synthesis in each cell cycle could lead to observed patterns of accumulation and doubling of certain enzymes in each cell cycle³. A cell size-related control over the time of doubling in rate of mRNA synthesis therefore offers the possibility of a widespread regulation of the synthesis of those enzymes not subject to specific controls at the transcriptional or translational levels.

We thank Laurence Errington and Robert Pitcairn for technical assistance and Tahia Benitez and Murdoch Mitchison

for constructive criticisms. The MRC and SRC provided financial support.

Received 24 October 1977; accepted 6 January 1978.

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letters to nature

Intensity of interstellar Lyman alpha

TARAFDAR and Wickramasinghe¹ suggested that the Lyman alpha (L α) intensity in the interstellar medium may be in the range $(1-4) \times 10^{-4}$ erg cm⁻² s⁻¹ sr⁻¹ and have pointed out that, if this is the case, L α may play an important part in photoemission from interstellar grains. Here we argue that typical L α intensities in interstellar H I are significantly less than 1×10^{-4} erg cm⁻² s⁻¹ sr⁻¹, considering three principal sources: photoionised H II regions; shock-heated gas; and collisional excitation and recombination in interstellar H I.

Approximately 50% of O stars and 80% of B0–B5 stars have, at any moment, escaped from their native cloud complex and generate diffuse ($n_e < 1$ cm⁻³) H II regions, and are estimated to emit H-ionising photons at a rate, projected onto the galactic disk, of 6.9×10^6 cm⁻² s⁻¹ in the solar neighbourhood², consistent with the galactic H α background³ if interpreted as recombination radiation⁴. If 0.7 L α photons result from each H recombination to level $n \geq 2$, then L α photons are generated in diffuse H II regions and injected into H I at a rate, projected onto the disk, of

$$j_{\alpha}(\text{H II}) = 5 \times 10^6 f_{\text{H II}} \text{ cm}^{-2} \text{ s}^{-1}, \quad (1)$$

where $f_{\text{H II}}$ is the fraction of L α photons which escape absorption by grains within the H II.

Shock-heated gas resulting from cloud–cloud collisions or supernova (SN) blastwaves will emit L α . If supernovae occur (in a 15 kpc disk) at a rate S , and a fraction ϵ of the initial energy E_0 per SN is converted, within the SN remnant (SNR), to L α , then the L α injection rate per disk area is

$$j_{\alpha}(\text{SN}) = 2.9 \times 10^6 \left(\frac{\epsilon}{0.2} \right) \left(\frac{S}{0.05 \text{ yr}^{-1}} \right) \left(\frac{E_0}{10^{51} \text{ erg}} \right) f_{\text{SN}} \text{ cm}^{-2} \text{ s}^{-1} \quad (2)$$

where f_{SN} is the fraction of L α which escapes absorption within the SNR. From Tamman's⁵ values for the total SN rate we estimate $S \leq 0.05 \text{ yr}^{-1}$ for the rate within the disk. The optically thin plasma in the interior of the SNR with $T > 10^5 \text{ K}$ contributes less than 0.01 to ϵ . If the ambient medium is initially neutral and then collisionally ionised by the blastwave shock, an appreciable number ϕ of L α photons per shocked H atom are emitted for shock speeds $v_s \gtrsim 40 \text{ km s}^{-1}$. Assuming initially neutral solar composition gas, Table 1 gives v_s and ϕ as a function of the shock temperature T_s .

Numerical models of SN blastwaves⁶ may be approximated by

the Sedov solution for $v_s \geq v_a$, an abrupt drop of v_s from v_a to v_b , and subsequent evolution with radius $r_s \propto t^{1/3}$. One then estimates, assuming $v_a = 200 \text{ km s}^{-1}$ and $v_b = 140 \text{ km s}^{-1}$,

$$\epsilon \approx 0.0237 \left(\frac{v_a}{200 \text{ km s}^{-1}} \right)^{-2} \left\{ 2v_a^2 \int_{v_a}^{\infty} v_s^{-3} \phi dv_s + v_b \int_0^{v_b} v_s^{-2} \phi dv_s \right\} \approx 0.17 \quad (3)$$

If the blastwave propagates into pre-ionised material ϵ will be much smaller. If $\epsilon \leq 0.2$ and $E_0 \leq 10^{51} \text{ erg}$, then

$$j_{\alpha}(\text{SN}) \leq 3 \times 10^6 f_{\text{SN}} \text{ cm}^{-2} \text{ s}^{-1} \quad (4)$$

Further conversion of SN energy to L α , by X-rays emitted (and cosmic rays accelerated) by the SNR will be included in $j_{\alpha}(\text{H I})$ below.

Either excitation by nonthermal particles or radiative recombination can produce L α in H I. For ionisation by either 100 eV X-rays⁷ or 2 MeV protons⁸ the number β of L α photons collisionally excited per H ionisation (both primary and secondary) can be estimated to be $\beta \approx 1.5$. The L α generation rate per volume is $(\beta + 0.7)n_H \xi$, where ξ is the total (primary and secondary) ionisation rate for H atoms due to either cosmic rays or soft X-rays; projected onto the plane this is

$$j_{\alpha}(\text{H I}) = 1.3 \times 10^6 \left(\frac{N_H}{6 \times 10^{20} \text{ cm}^{-2}} \right) \left(\frac{\langle \xi \rangle}{10^{-15} \text{ s}^{-1}} \right) \text{ cm}^{-2} \text{ s}^{-1} \quad (5)$$

where N_H is the full-thickness H I column density normal to the plane. As the average $\langle \xi \rangle$ is almost certainly less than 10^{-15} s^{-1} , and $N_H \approx 6 \times 10^{20} \text{ cm}^{-2}$ (ref. 9), evidently $j_{\alpha}(\text{H I}) < 1.3 \times 10^6 \text{ cm}^{-2} \text{ s}^{-1}$.

Table 1 The number ϕ of L α photons per shocked H atom

$T_s(\text{K})$	$v_s(\text{km s}^{-1})$	ϕ	$T_s(\text{K})$	$v_s(\text{km s}^{-1})$	ϕ
5×10^6	416	1.1	1.5×10^5	72	2.6
2×10^6	263	1.1	1×10^5	59	1.8
1×10^6	186	1.3	7×10^4	49	1.2
7×10^5	156	1.4	5×10^4	42	0.86
5×10^5	132	1.7	3×10^4	32	0.44
3×10^5	102	2.8	2.5×10^4	29	0.34
2.5×10^5	93	4.2	2×10^4	26	0.23
2×10^5	83	3.4	1.5×10^4	23	0.12

From equations (1), (4) and (5) the total $L\alpha$ injection rate into H I is

$$j_{\alpha}(\text{total}) < 10 \times 10^6 \text{ cm}^{-2} \text{ s}^{-1} \quad (6)$$

It will be argued that $f_{\text{H I}} \approx 0.6$, so that the inequality in equation (6) seems reasonably secure. Assuming no escape from the disk; $N_{\text{H}} = 6 \times 10^{20} \text{ cm}^{-2}$; a grain absorption cross section per H nucleus $\sigma = 0.7 \times 10^{-21} \text{ cm}^2$ at $\lambda = 1,215 \text{ \AA}$ (refs 10–12), the mean $L\alpha$ intensity is estimated from equation (6) to be

$$\langle I_{\alpha} \rangle = \frac{h\nu j_{\alpha}}{4\pi n_{\text{H}} \sigma} \text{ sr}^{-1} < 3.1 \times 10^{-5} \text{ erg cm}^{-2} \text{ s}^{-1} \text{ sr}^{-1} \quad (7)$$

or less than 1.9×10^6 photons $\text{cm}^{-2} \text{ s}^{-1} \text{ sr}^{-1}$. The continuum background near 10.2 eV has been estimated^{13,14} to be about 1.9×10^6 photons $\text{cm}^{-2} \text{ s}^{-1} \text{ sr}^{-1} \text{ eV}^{-1}$, so that $\langle I_{\alpha} \rangle$ is at most comparable to the ultraviolet continuum in a 1 eV band centred on 10.2 eV.

Having estimated the mean $L\alpha$ intensity, we investigate its spatial variation. As seen above, $\sim 50\%$ of the $L\alpha$ generation occurs in diffuse H II regions, so consider, as a typical example, the Strömgren sphere surrounding an O7 V star emitting $N_{\text{e}} = 7.2 \times 10^{48}$ H-ionising photons s^{-1} (ref. 15) in a cloudless medium with $n_{\text{H}} = 0.5 \text{ cm}^{-3}$, $T = 8,000 \text{ K}$, and dust absorption cross section/H $1.1 \times 10^{-21} \text{ cm}^2$ and $0.7 \times 10^{-21} \text{ cm}^2$ for Lyman continuum and $L\alpha$, respectively. For a dusty Strömgren sphere¹⁶ the radius at which $n(\text{H}^+) = n(\text{H}^0)$ is $R_{1/2} \approx 87 \text{ pc}$, the $L\alpha$ optical depth (from $r = 0$ to $r = R_{1/2}$) at line centre is 9×10^4 , while the $L\alpha$ optical depth in dust is 0.094. Formulae fitted¹⁷ to numerical calculations predict an escape probability $f_{\text{H I}} \approx 0.57$ and a typical frequency shift for the escaping photons of ~ 4.0 Doppler widths $= 46 \text{ km s}^{-1}$. In the surrounding H I the escaping photons are on the extreme Lorentz wing, have a mean free path $= 0.26$ ($\text{cm}^{-3}/n_{\text{H}}$) pc, scatter about 1,800 times before being absorbed, and diffuse about $0.52(1,800)^{1/2}$ pc away from the boundary of the H II region. The mean $L\alpha$ intensity in this zone of thickness $\Delta R = 22 \text{ pc}$ is

$$I_{\alpha} \approx \frac{0.7 f_{\text{H I}} N_{\text{e}} h\nu}{16\pi^2 n_{\text{H}} \sigma R_{1/2}^2 \Delta R} \approx 3.0 \times 10^{-4} f_{\text{H I}} \text{ erg cm}^{-2} \text{ s}^{-1} \text{ sr}^{-1} \quad (8)$$

The leading dependence of I_{α} is $I_{\alpha} \propto f_{\text{H I}} n_{\text{H}}^{4/3} N_{\text{e}}^{1/3}$. As a second example consider a spherical SNR with $E_0 = 10^{51} \text{ erg}$ in a medium with $n_{\text{H}} = 0.5 \text{ cm}^{-3}$ and with shock speed $v_s = 100 \text{ km s}^{-1}$ (and radius $R \approx 33 \text{ pc}$ (ref. 6)); similar estimates lead to an average $L\alpha$ intensity $I_{\alpha} \approx 2 \times 10^{-4} \text{ erg cm}^{-2} \text{ s}^{-1} \text{ sr}^{-1}$ in a 35 pc thick shell surrounding the SNR.

The following conclusions are reached. First, the mean $L\alpha$ intensity in galactic H I is at most about $3 \times 10^{-5} \text{ erg cm}^{-2} \text{ s}^{-1} \text{ sr}^{-1}$. Second, the $L\alpha$ energy density due to H II regions and supernovae is very patchy, varying over length scales of $\sim 10 \text{ pc}$ or so. Third, within zones of enhanced $L\alpha$ (perhaps 10% of galactic H I) surrounding supernovae and H II regions the $L\alpha$ intensity may be large compared to the continuum background in the 10.2–13.6 eV band. Finally, in most galactic H I the energy density of $L\alpha$ is small compared to that of continuum ultraviolet.

This work was partially supported by NSF grant AST-75-21153.

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Received 3 November; accepted 14 December 1977.

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Search for high energy γ -ray bursts from evaporation of primordial black holes

PRIMORDIAL black holes (PBH) have been predicted by Hawking¹. Density fluctuations in an early Universe might have given rise to a power law spectrum of low mass black holes which would evaporate by a thermal radiation process. Holes of initial mass $< 10^{12} \text{ kg}$ formed at the beginning of the Universe would have evaporated by now. However, holes of initial mass $> 10^{12} \text{ kg}$ might now be entering the terminal stages of evaporation, culminating in an explosion. The total energy liberated in particle emission and the corresponding radiation time scales depend critically on the nuclear models adopted². The composite particle (CP) model predicts the liberation of 10^{27} J during the final 10^{-7} s of the explosion. About 10% of the total energy goes into γ rays with the spectrum peaked at 250 MeV. The elementary particle (EP) model predicts the liberation of 10^{30} photons during the final 0.1 s of the explosion at energies around $5 \times 10^{12} \text{ eV}$ (refs 3, 4). We report here the results of a search for γ rays associated with the evaporation of PBHs, and upper limits are presented based on the latter model.

Two ground based extensive air shower detection systems, separated by 250 km, have operated in time coincidence since January 1975. The detection systems have been described in detail elsewhere^{5,6}. Four scintillation counters are used at each station to detect showers. The counter thresholds are set at the single particle level. The outputs of the four individual detectors are added together to give an event rate which varies between 780 and 820 s^{-1} , at each station. Such events are termed singles. Threshold measurements show that for this mode 50% of the showers are in the energy range $1.5\text{--}9 \times 10^{13} \text{ eV}$. In addition, at each station, coincident combinations of at least two out of the four counters are formed, giving a rate of typically 3 s^{-1} . Such events are termed multiple, with corresponding shower energies in the range $7\text{--}20 \times 10^{13} \text{ eV}$.

Overflow scalars individually monitor the single and multiple event rates over a range of time scales. If the preset count is exceeded during any sampling interval, the absolute time of occurrence is recorded to an accuracy of 10^{-3} s . Details of the preset levels and associated sampling intervals are shown in Table 1. Event timing is accomplished by a 10 kHz waveform, derived from a 5 MHz oven controlled crystal and fed to a 7-decade digital logic scalar with latch facilities. Absolute time from the 60 kHz MSF Rugby transmission is injected into the system each hour to produce an absolute time marker. For each event, 32 bits of timing and coding information are stored in a 1,024-bit buffer memory. The overall writing time for any event into the memory is 8 μs . On completion of storage of the 32nd event, a readout sequence is initiated and the buffer contents are punched on paper tape. The total readout time is 5 s, which represents the system dead time per buffer of 32 events. Both stations have accumulated data almost continuously since January 1975.

The analysis procedure adopted has involved a computer search of the tape records containing the coded events recorded at each station. Tests with artificial events injected simultaneously at both stations showed that both the hardware and software functioned as designed. Because of the systems threshold energy for shower detection, our observational data can only test the EP model as a possible mechanism for the production of exploding PBHs. We make the assumption that any PBH close enough to Earth will produce a flux of high energy γ rays at the top of the

Table 1 Results of an average 24-h observation

Sampling time interval	Preset count level	Event code type	Event classification	Predicted rate (d^{-1})	Average observed rate (d^{-1})
10^{-2} s	26	2	Single	2.9	3
10^{-1} s	124	3	Single	2.5	2
1 s	12	5	Multiple	6.1	5

Table 2 Upper limits for different characteristic time scales

Code pair	Coincidence resolving time (τ) (s)	Total search time (T) (d)	No. of coincidences observed in time $T(N_o)$	No. of coincidences predicted on a random basis in time $T(N_r)$	Upper limit to the detectable γ -ray burst rate per year (N_b)	Upper limit to the rate of PBH explosions per unit volume (N_{exp}) ($\text{pc}^{-3} \text{yr}^{-1}$)
(2-2)	10^{-2}	802	0	0.0005	2.1	4.8×10^3
(3-3)	10^{-1}	802	0	0.05	2.1	1.9×10^4
(5-5)	1	821	0	0.3	2.0	6×10^3

atmosphere which in turn initiates cascades of showers of basically the same temporal characteristics at the two stations. Such considerations dictate that in the analysis, no cross coded coincidences (Table 1) should be looked at. The choice of coincidence resolving time is determined by the predicted characteristic time of the radiation burst for the EP model, 0.1 s. In view of the probable uncertainties in this model, however, we have looked for coincidences between like coded events in the range of time scales from 0.01–1 s. Table 2 summarises the results of this analysis. It tabulates both the observed (N_o) and random (N_r) expectation rates for different resolving times (τ) pertaining to total search times (T).

The number of observed coincidences (N_o) was used to calculate upper limits, at the 99% confidence limit, of the detectable γ -ray burst rate (N_b) for the different resolving times (τ). Based on the EP model, upper limits to the density of PBH explosions (N_{exp}) within the sensitive volume of the detectors field of view have been determined as follows.

The model predicts the emission of 10^{30} photons at energies around 5×10^{12} eV. In view of uncertainties in the model and for the calculation we assume that these 10^{30} photons will be detectable within both ranges of energy resolution of the detection systems. The shower collection areas appropriate to both these energy ranges are 1,000 m² for the multiple coded events and 2,500 m² for those coded as singles. The larger collection area in the lower energy band reflects a lower particle density requirement in the detector trigger system. The field of view of each stations detection system is estimated to be 1 sr. As an example, consider the estimation of (N_{exp}) for a code 5 coincidence where on average the trigger requirements at each station demand an excess of 9 counts per s. The maximum distance to which an exploding PBH would still remain detectable would be a radius of 3×10^{15} m. The corresponding sensitive volume within the field of view is $3.3 \times 10^{-4} \text{pc}^3$; hence, for an upper limit of 2.0 bursts yr^{-1} , we estimate an upper limit to the number of explosions (N_{exp}) of $6 \times 10^3 \text{PBH pc}^{-3} \text{yr}^{-1}$. Values of (N_{exp}) for the other time scales τ are given in Table 2.

A significantly better upper limit to the rate of explosion of PBHs leading to γ -ray bursts has been given by Porter and Weekes^{7,8} but this is based on the CP model in which 10^{27} J is released in the explosion. More recently, Rees⁹ has suggested that if PBHs exist and if they emit particles, then they might be detectable indirectly from radio and optical emission arising from the interaction of the charged particles with the ambient interstellar magnetic fields. Upper limits to the possible pulsed radio emission based on Rees have been given by Jelley *et al.*¹⁰ while Porter and Weekes¹¹ quote limits to the rate of emission of optical pulses.

This research was partly sponsored by the National Science Council of Ireland.

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Received 9 November 1977; accepted 12 January 1978.

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Indentation hardness and semiconductor-metal transition of germanium and silicon

A HARD indenter pressed into the surface of a ductile solid such as a metal will produce a permanent indentation. The mean pressure over the indentation is known as the hardness of the solid and is a measure of its yield or flow stress¹. With many relatively brittle materials such as glass and rock salt, it is possible to form plastic indentations at low loads. This is because the indentation process involves a very large component of hydrostatic stress which inhibits brittle failure: the material then yields by flow and the hardness is again a measure of the (ductile) yield stress of the solid²⁻⁴. At high loads the indentation is usually accompanied by appreciable cracking. Crack-free hardness indentations may also be made at low loads in single crystals of germanium and silicon (C. A. Brookes and our unpublished data) and it is suggested here that this may be due at least in part, to a different mechanism.

Germanium has fourfold coordination and is a semiconductor. Under a hydrostatic pressure of approximately 115–120 kbar the crystal structure changes: there is six-fold coordination and the germanium becomes metallic⁵. Shimomura *et al.*⁶ give the transition pressure for polycrystalline germanium as 100 kbar. We now note that the indentation hardness of germanium, although somewhat dependent on crystal face and indenter orientation, is of order 800 kg mm⁻², that is, 80 kbar, which is comparable with the transition pressure. Silicon shows a similar behaviour. The semiconductor-metal transition occurs at a pressure of 190 kbar (ref. 5) or for polycrystalline silicon, 155 kbar (ref. 6); the indentation hardness is of order 120 kbar. Thus both the transition pressure and the hardness value are again comparable and are about 50% greater for silicon than for germanium.

We may now consider the corresponding behaviour of diamond. Hardness indentations may be made, though they are always apparently accompanied by some cracking. The hardness value is of order 10,000 kg mm⁻², suggesting a metallic transition at a pressure of about 1,000 kbar. Such a conclusion is supported by direct observations on the metallic transition of diamond by Vereshchagin *et al.*⁷. These results suggest that the indentation hardness is indeed close to the semiconductor-metallic transition pressure and that under conditions of hardness measurements, the material around the indenter becomes sufficiently ductile to sustain plastic flow.

Table 1 Correlating bond energy gaps with transition pressures

Solid	a (nm)	E_R (eV)	E_R/a^3 (eV nm ⁻³)	Transition pressure (kbar)	Indentation hardness (kbar)
Ge	0.566	4.3	24	120 (100)	80
Si	0.543	4.8	30	190 (155)	120
C	0.356	13.5	300	~1,000	1,000

The average bond energy gaps (E_g) for Ge, Si and diamond are 4.3, 4.8 and 13.5 eV respectively⁶. It is interesting to consider whether they are related to the transition pressures. Clearly, on dimensional grounds, it is not possible to correlate energies with pressures; one needs energy divided by volume. For simplicity we use the cube of the lattice spacing a . The results are given in Table 1.

In view of the calibration uncertainties in high pressure experiments, the agreement seems reasonable. The figures indeed suggest that the transition pressure and true indentation hardness of diamond should be somewhat greater than 1,000 kbar. It would be interesting to know how far such correlations apply to other types of solids which show a semiconductor-metallic transition under pressure. It may turn out that in some systems the bond strength is a more relevant parameter than the average bond energy gap.

We thank Sir Nevill Mott, Dr E. A. Davis and Dr A. D. Yoffe for helpful discussions and Dr C. A. Brookes for additional data on microhardness. We are indebted to the referee for bringing to our attention the paper by Vereshchagin *et al.*⁷.

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Received 26 October; accepted 20 December 1977.

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Evidence of photochemical haze in the atmosphere of Greater London

PHOTOCHEMICAL pollution in Greater London, as evidenced by unnaturally high ozone concentrations, has been reported on several occasions¹⁻⁵. In Los Angeles, where photochemical activity has been experienced for many years, an obvious feature of this pollution is aerosol formation, which has been attributed to the participation of SO_2 in the photochemical reaction sequence, with subsequent formation of sub- μm sulphate particles⁶. Although SO_2 concentrations are higher in Greater London, there have been no reported measurements of photochemical haze, but Cox and Penkett⁷, by extrapolation of laboratory measurements of air samples from Harwell, have suggested that a conversion rate of $10\% \text{ h}^{-1}$ for SO_2 to sulphate aerosol may be possible in Greater London; this would be sufficient to produce large quantities of sulphate aerosol. Identification of such processes is important because evidence suggests that photochemically produced sulphate aerosols can, in certain circumstances, account for a large proportion of total particulate mass in the $0.1\text{--}1.0 \mu\text{m}$ size range⁸. This size range is important in determining the optical scattering coefficient of polluted air⁹, and hence visibility, and potentially as a respiratory tract irritant because of its ability to penetrate deeply, in combination with its chemical nature¹⁰. We report here preliminary observations based on nephelometric measurements of the optical scattering coefficient of air over central London during June to August 1976, and evidence of haze formation in the London area as deduced from the Daily Weather Reports (DWR) of the Meteorological Office (MO), and show how these relate to the occurrence of photochemical processes over Greater London.

Throughout the summer of 1976, the optical scattering coefficient, b_{scat} , and concentrations of various gaseous pollutants, including ozone, were measured at the Greater London Council's (GLC) County Hall roof-top site in central London. b_{scat} was measured by a broad-band integrating nephelometer with a quoted effective wavelength of 500 nm, and in which incoming air was heated to $\sim 20^\circ\text{C}$. Ozone was monitored by chemiluminescent reaction with ethylene. The optical scattering coefficients can be related, at least approximately, to meteorological range (visibility), L_v , by Koschmieder visibility theory¹¹, such that $L_v \approx 3.9 b_{\text{scat}}^{-1}$, where scattering, as opposed to absorption, is the dominant factor in atmospheric extinction. Figure 1 shows the 24 h mean value of b_{scat} plotted against the maximum 1-h mean ozone concentration for each 24 h period, noon-to-noon, during the 3 months, June, July and August. Expressing b_{scat} as a linear function of the ozone concentration by the least-squares method yields a correlation coefficient of 0.7, suggesting that a large fraction of the variance of b_{scat} could be associated with photochemical pollution. Further understanding of the relationship of higher values of b_{scat} to photochemical events, as evidenced by unnatural ozone concentrations, requires detailed examination of individual episodes.

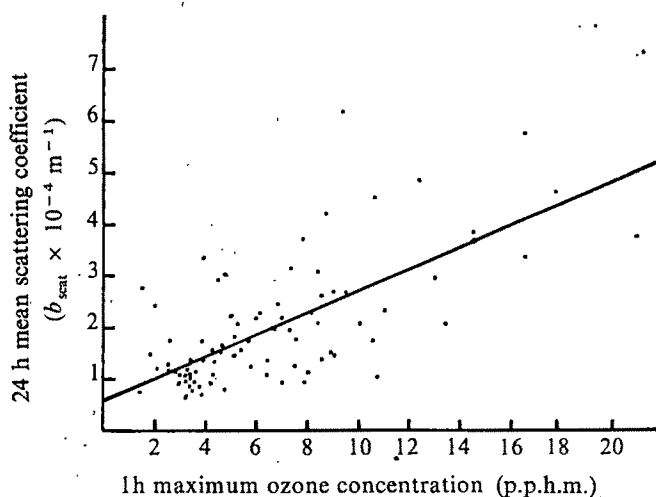


Fig. 1 24-h mean values of the optical scattering coefficient, b_{scat} , plotted versus 1-h maximum ozone concentrations as recorded at the GLC County Hall site in central London for each 24-h period starting at 1200 h GMT during May to August 1976. Ozone concentrations are reported as parts per hundred million (v/v). Line through data points is the least-squares fit, with intercept 0.6 ± 0.4 , and slope 0.21 ± 0.06 , where errors correspond to 2σ . $r = 0.69$ with 82 data points.

Of particular interest is the 0600 h record of the 4-times daily DWR for 28 June. At this time a dense haze is reported from each of three MO stations at Heathrow, Kew and Gatwick, with respective visibilities of 1.8, 1.3 and 1.5 km^{12} , as measured by Gold extinction meters. The haze was denser at these sites, which lie respectively 21 km west-southwest, 13 km west southwest and 40 km south of central London, than at the seven other MO reporting stations elsewhere in south-east England, and represented the densest haze reported from any of the MO observation stations listed for Britain in the DWR during the entire June to August period¹².

Ozone, b_{scat} , and some relevant meteorological parameters are shown for this period, for central London, in Fig. 2. The ozone concentrations, which reached record reported levels for a UK city on 26 and 27 June, follow the usual diurnal pattern, while aerosol formation and growth, as measured by b_{scat} , shows an apparent time lag of some hours behind peak ozone concentrations. Time differences of this nature have also been observed in smog chamber simulations¹³, and during ambient

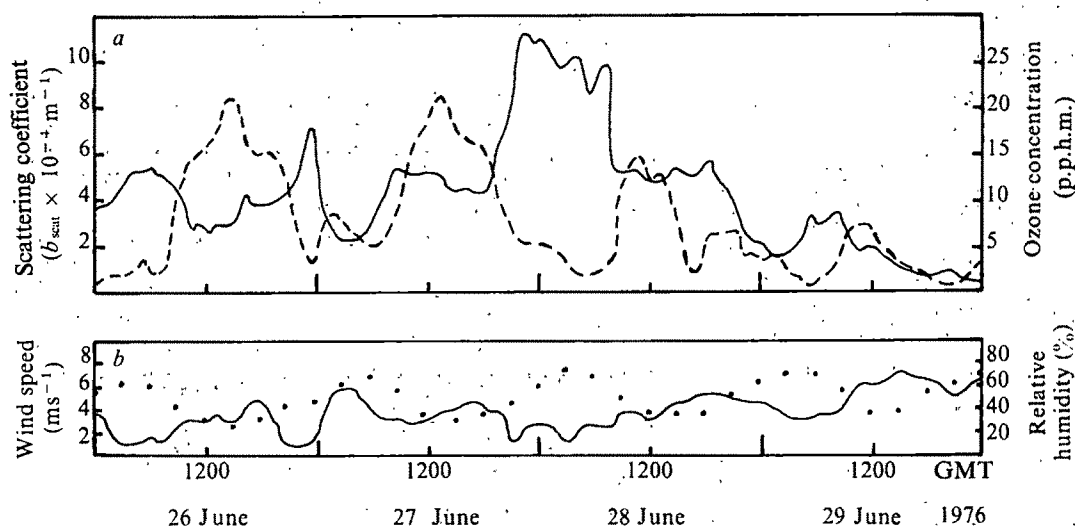


Fig. 2 Values of the optical scattering coefficient and ozone concentrations as recorded at the GLC County Hall site from 26 to 29 June 1976, together with meteorological data from the London Weather Centre. *a*, Solid line is b_{scat} ; dashed line is ozone; *b*, solid line is wind speed; dotted line is relative humidity.

air measurements of ozone and photochemical aerosol formation in studies carried out in the USA¹⁴. Bearing in mind the light east southeast surface wind, of speed $\sim 2 \text{ m s}^{-1}$, during the night of 27–28 June, and the calm conditions aloft (Balthum data from MO station, Cardington, Bedfordshire), it is not unreasonable to assume that the dense haze reported at the MO Heathrow and Kew sites is of the same origin as the b_{scat} peak detected at County Hall. Because ozone concentrations were higher in central London on 27 June than values reported from elsewhere in south-east England⁴, and because meteorological conditions were suitable for the generation and detection of locally produced ozone over central London on that day, we have proposed elsewhere that a substantial portion of the ozone detected at County Hall at this time may be formed from locally emitted precursors⁵. Similarly, the detection of the unusually dense haze at the downwind MO stations closest to Greater London a few hours after the unprecedented ozone concentrations is suggestive of a photochemical formation mechanism which is, in part, attributable to precursors emitted in or close to the Greater London area. During the remainder of the episode illustrated in Fig. 2, solar radiation was still high, and thus conducive to further photochemical activity. However, it is considered that the brisker, although still easterly, winds would have swept precursors into downwind areas before significant quantities of ozone or aerosol had been formed.

Table 1 records other instances during the study period, when visibility at Heathrow or Kew, as reported by the MO¹², was restricted by haze to $< 5 \text{ km}$, and was also less than at the other

MO stations in south-east England, days on which mist or fog occurred at some locations having been eliminated. Visibility degradation at Heathrow and Kew, as enumerated in the DWR, was generally highest at the 0600 h, or occasionally at the 0000 h GMT reporting times. With the possible exception of 5 June, all 24-h periods listed in Table 1 experienced light winds from the eastern sector which could have advected aerosols from the Greater London conurbation to these locations. In addition, maximum 1-h mean ozone concentrations exceeded 8 p.p.h.m. at County Hall, or elsewhere in Greater London⁵, on 80% of these occasions. Ozone concentrations of this magnitude usually indicate a significant amount of photochemical activity.

Preliminary examination of other 24-h periods which did not have significantly more haze at Heathrow or Kew than elsewhere in south-east England, but during which ozone concentrations exceeded 8 p.p.h.m. in Greater London, shows that some coincide with periods of westerly winds, while others may be associated with higher wind speeds or lower relative humidities. It is also evident that, on some occasions, high ozone concentrations in the London area are not due to local photochemical activity, but to transportation from remote sources^{5,15}.

We thank Dr M. J. R. Schwar for discussion, and R. T. Kelly, Scientific Adviser to the GLC, for permission to publish this work. The views expressed do not necessarily represent those of this Council.

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Received 29 June; accepted 22 December 1977.

Table 1 Minimum visibilities at Heathrow and Kew

	Visibility (km)		Maximum 1-h mean ozone concentration (p.p.h.m.)	1800– 0600 h wind speed (ms^{-1})	1800– 0600 wind direction
	Heathrow	Kew			
4 June	3.0	4.8	7.0	1.9	Variable
7 June	4.5	3.0	11.0	4.6	90
8 June	2.0	4.8	11.9	2.2	90
27 June	1.8	1.3	21.2	2.4	80
3 July	4.2	5.0	17.8	3.3	70
5 July	3.5	3.0	13.0	5.3	60
12 August	3.8	5.6	12.6	2.3	130
14 August	5.6	2.8	5.9	4.4	40
17 August	5.0	4.0	10.2	3.6	80
19 August	5.0	2.0	8.9	3.7	40

Data for the 24-h periods starting at 1200 h GMT on the day given, as reported in the DWR, together with maximum 1-h mean ozone concentrations recorded in Greater London, and nocturnal average wind speed and direction from the central London Weather Centre, for the same period.

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Volcanic dust and changes in Northern Hemisphere temperature

RADIATION calculations which seemed to demonstrate that changes in the temperature of the Northern Hemisphere on a wide variety of time scales could be attributed to changes in the amount of volcanic dust in the atmosphere have been reported by Pollack *et al.*¹ In particular, they claimed that the observed average temperature depression of 0.3 °C two years after major volcanic eruptions agreed with their calculated value and that the clearance of volcanic dust after the frequent strong eruptions at the end of the nineteenth century was responsible for a rise in the Northern Hemisphere temperature of 0.5 °C between the decades starting 1880 and 1935. Here we look critically at these two claims in the light of the known noisiness of meteorological data and the uncertainties in the estimates of volcanic dust in the atmosphere.

For the observed changes following major eruptions Pollack *et al.*¹ used values given by Bray². These were for the years following all volcanic eruptions since 1750 for which Lamb³ had estimated the total dust veil as 1,000 units or more. Lamb⁴, however, gave revised estimates of the dust veil index (DVI), and a selection of years on the same criterion (DVI ≥ 1,000) results in three changes from Bray's list. The original and altered dates (in brackets) are: 1752, 1766, 1775, 1783, 1803 (1805), 1807 (1809), 1815, 1831, 1835, 1846, 1878 (1875), 1883, 1902.

Average temperature anomalies in the years following the two selections are shown in Table 1. The temperature anomalies for the individual years were obtained from data by Arakawa⁵. They are estimates for the Northern Hemisphere begun by Koeppen and continued by Humphreys up to 1921. After 1811 the basis of the temperature series was improved by the inclusion of some tropical locations. Thus the temperature anomalies for the major eruptions after 1811 in Bray's selection have been worked out and are given in Table 1. Bray based his claim that the temperatures for years +1 and +2 were reduced by volcanic eruptions on the fact that the number of positive departures in those years was well below that to be expected by chance (about 1 in 100 for year +1 and 9 in 100 for year +2). In the revised selection there is still a small probability of the result being due to chance for year +1 but for year +2 the probability of a chance result is as high as 1 in 5. As far as the value for year +2 is concerned, the reality of the result apparently depends on the selection of years.

It may be desirable to examine the reality of the results using the magnitude as well as the sign of the anomalies. To test the significance of the residuals in Table 1 we need to estimate the standard error of samples of this size. The standard deviation of the yearly anomalies of the Koeppen-Humphreys series from 1750 to 1920 with the exclusion of the five years following each of the 13 major eruptions is 0.50 °C. Thus averages of 13 values chosen at random from this series can be expected to have a standard deviation from zero of 0.14 °C. It is therefore very likely that the value of -0.27° for year +2 to which Pollack *et al.*¹ refer contains a substantial sampling error. The values of

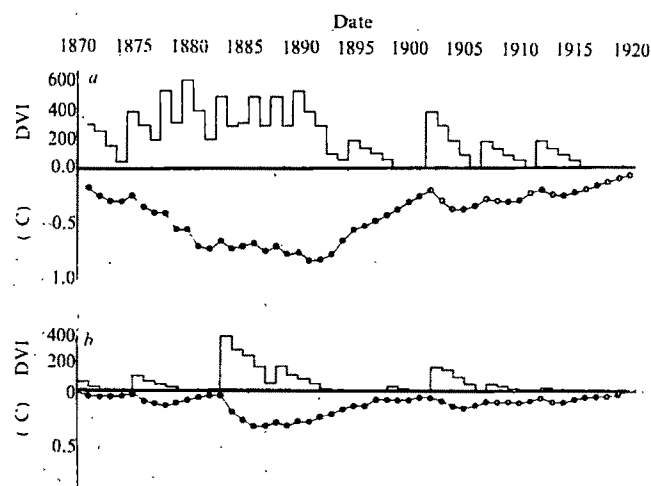


Fig. 1 Calculated temperatures for two sets of DVI. *a*, All eruptions mentioned by Lamb; *b*, well substantiated eruptions ref. 4 Table 7a.

-0.13 and -0.06 in Table 1 could just as well be taken as the effect of volcanic dust.

The first part of their claim seems to rest, therefore, on rather shaky ground—the observed temperature depression for year +2 is apparently nearer -0.15 ± 0.14 °C. If we suppose that there is some depression of temperature following major eruptions but that it is well below 0.5 °C, then for the second part of their claim to be valid there must have been a concentration of strong eruptions and enough persistence of the temperature depression following each to bring the hemispheric temperature 0.5 °C below the clear atmosphere equilibrium level for several years. We now use a simple mathematical model to see if this could have been the case.

The solar radiation reaching the earth's surface is given as S in the original state and $S(1-\alpha)$ when volcanic dust is present in the upper atmosphere, with corresponding equilibrium temperatures T_0 and T_0^1 . Following Budyko⁶, we assume the net outgoing longwave radiation from the surface to be a linear function of T when global averages are being considered, so that $S = aT_0 + b$ and

$S(1-\alpha) = aT_0^1 + b$, and $\Delta T_E = T_0 - T_0^1 = S\alpha/a$. The change between the two equilibrium temperatures is described by

$$\frac{CdT}{dt} = S(1-\alpha) - aT - b, \quad T = T_0 \text{ when } t = 0$$

where C is the equivalent heat capacity of the global surface layer. This leads to

$$\Delta T = \Delta T_E (1 - \exp(-kt)) \quad (1)$$

where $\Delta T = T_0 - T$ and $k = a/c$. Taking the data from Table 1 of Pollack *et al.*¹ it is apparent that, to a first approximation, ΔT_E for their calculation can be written $[8(\Delta\tau)]$ °C where $\Delta\tau$ is the change in optical depth due to the volcanic dust. For an average cloudiness of 50%, Budyko⁶ gives $a = 0.09$ kcal cm⁻² month⁻¹ °C⁻¹. The appropriate value of C is difficult to define precisely, but a change over a few decades is likely to be determined primarily by the depth of the ocean whose temperature is significantly affected by the temperature change. Assuming, as Schneider and Mass⁷ did, that this depth is about 75 m, and taking into account the fact that the oceans cover 71% of the earth's surface, an appropriate value for C is 5.3 kcal cm⁻² °C⁻¹. This gives a relaxation time for the surface temperature of C/a which is nearly 60 months, and of the same order as that apparently used by Pollack *et al.* and Oliver⁸ in a similar treatment of the effects of volcanic dust.

Table 1 Temperature anomalies (°C) after major eruptions

Years after eruption	0	+1	+2	+3	+4	+5
Bray's selection	+0.08	-0.31	-0.27	-0.07	+0.15	-0.28
Number of positive departures	8	2	4	6	8	5
Revised selection	-0.04	-0.29	-0.13	-0.20	+0.03	-0.23
Number of positive departures	6	2	6	4	6	5
Major eruptions after 1811	-0.01	-0.30	-0.06	-0.03	-0.03	-0.21

Table 2 Two possible Sequences of annual total DVI after Lamb

DATE	1878	79	80	81	82	83	84	85	86	87	88	89	90	91	92	93
All eruptions	550	320	620	400	200	500	300	320	510	300	490	280	550	400	275	100
Well-substantiated eruptions*	30	0	0	0	0	400	300	240	170	50	170	125	85	45	20	15

*From ref. 4 Table 7a.

Table 3 Calculated and observed temperature anomalies for 5-yr periods in °C

		1870-74	1875-79	1880-84	1885-89	1890-99	1895-99	1900-04	1905-09	1910-15	1915-20	1920-24
Calculated	All eruptions	-0.20	-0.04	-0.67	-0.72	-0.76	-0.46	-0.27	-0.32	-0.24	-0.16	-0.05
	Well-substantiated eruptions	-0.03	-0.07	-0.07	-0.29	-0.22	-0.09	-0.07	-0.11	-0.08	-0.05	-0.01
Observed	Koeppen-Humphreys	-0.49	-0.18	-0.23	+0.11	+0.05	+0.17	+0.25	0.00	0.09	0.00	N/A
	Mitchell-Budyko	+0.05	+0.03	-0.13	-0.13	-0.06	+0.10	+0.07	0.00	0.04	0.10	0.20

Equation (1) can be recast in the form

$$\delta(\Delta T) = k[\Delta T - \Delta T_E]\delta t \quad (2)$$

$\delta(\Delta T)$ is the change in temperature during a time interval δt where the temperature depression is ΔT and the equilibrium depression is ΔT_E . Pollack *et al.*¹ have assumed that eruptions of the magnitude of Krakatoa produce a change in optical depth of 0.1, and Lamb⁴ supposes that the DVI for the first year after such an eruption is 400 units. Accordingly we suppose ΔT_E for each year is given by $0.8 \times \text{DVI}/400$ and equation (2) using time steps of one year becomes

$$\delta(\Delta T) = 0.2 [\Delta T - (0.8 \times \text{DVI}/400)] \quad (3)$$

There was a sequence of strong volcanic eruptions during 1878-93 and two sets of total annual DVI values for this period taken from Lamb⁴ are given in Table 2.

The calculated temperature anomalies from equation (3) for the period 1878-1920 for two such sets of data extended to 1920 are shown in Fig. 1. Five-year mean temperature anomalies for the two sets are given in Table 3, together with values from the Koeppen-Humphreys and the Mitchell-Budyko series of Northern Hemisphere temperatures. The latter are expressed as anomalies from the mean for 1870-1921, which makes them comparable with the former series.

The calculations indicate that the maximum depression of temperature due to volcanic dust may be expected in the decade 1885-94 and that with the assumed relaxation time, temperature would be back to the clear atmosphere value by 1920. The calculated and observed changes of temperature over this interval are shown in Table 4.

The value for all eruptions seems to be too large: that for the well-substantiated eruptions is reasonable when compared with the Mitchell-Budyko series, the ones usually accepted in climatic change studies. It is generally agreed that there was very little, if any, volcanic dust in the atmosphere after 1920 until the late 1940s at the earliest. Humphreys⁹ remarks that by July 1914, to judge from the pyrohelioelectric data, the dust from the Katmai (Alaska) eruption in 1912 had fully passed and the measurements remained steady to 1940. Thus it is improbable that any of the

warming after 1920 can be attributed to the volcanic effect. From Table 4 this puts a likely upper limit of 0.2 °C on the contribution of volcanic dust to the climatic warming between the end of the nineteenth century and the 1940s.

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Received 29 July; accepted 15 December 1977.

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Volcanic dust influence on glacier mass balance at high latitudes

EXPLOSIVE eruptions, which inject large quantities of volcanic dust into the earth's upper atmosphere, are believed to be important factors in climatic change. Theoretical considerations suggest that the greatest climatic effect of a stratospheric dust veil would be at high latitudes during summer months, when solar radiation passes through the greatest depth of atmosphere and the surface is illuminated continuously¹. Furthermore, the residence time of volcanic dust is greatest at high latitudes, where it may remain in the upper atmosphere for a decade or more, depending on particle size and initial injection height². Here we present evidence that the eruption of Mount Agung (8 °S, 115 °E) in March 1963, was responsible for a marked change in the climate of the North American High Arctic and that this change has had a significant impact on glacier mass balance in the region.

The North American High Arctic (north of lat 74 °N) contains the greatest concentration of land-based snow and ice outside Greenland and Antarctica, with glaciation levels in some areas <300 m above sea level³. The ablation, or melt, season averages 86-123 d yr⁻¹ near sea level and up to 53% of annual precipitation may occur during this period⁴. At higher elevations, the ablation season is even shorter and all summer precipitation falls as snow, increasing surface albedo and continuously retarding the melt process at the glacier or ice cap surface. Because this season is so brief, a change in

Table 4 Calculated and observed temperature changes 1885-94 to 1915-19

		°C
Calculated	All eruptions	+0.58
	Well substantiated eruptions	+0.20
Observed	Koeppen-Humphreys	-0.08
	Mitchell-Budyko	+0.20

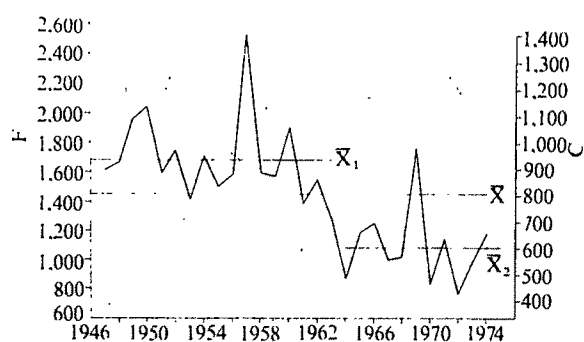


Fig. 1 Annual $(T_x - T_n)$ MDD totals at Thule, northwestern Greenland, showing the abrupt decrease in overall 'summer warmth' after the eruption of Mount Agung in 1963.

summer climate has significant impact on glacier mass balance in the region.

An abrupt and significant change in the summer climate of the Canadian Arctic occurred between the summer of 1963 and 1964 and the resulting conditions have persisted^{4,5}. Mean July freezing-level heights in the atmosphere in the decade after 1963 were up to 500 m lower than in the preceding decade. At the surface, mean maximum July temperatures since 1963 have averaged 1.1 °C to 2.7 °C lower than in the pre-1963 period. This fall in temperature was partly brought about by a change in the frequency of extremely warm summer days. At Alert, for example, there was an average of 2.3 d yr⁻¹ with maxima >15.5 °C (60 °F) between 1950 and 1963; between 1964 and 1976 there have only been four such days⁴.

Particularly useful indices of the change in total summer 'warmth' are annual melting degree day totals over the past 25–30 yr. A melting degree day (MDD) is the difference between 0 °C and daily maximum (T_x MDD) or minimum (T_n MDD) temperatures, when the latter are above 0 °C. Melting degree day totals have fallen significantly throughout the High Arctic since 1963 (Table 1). This is most apparent at Thule, Greenland (Fig. 1), where mean MDD totals 1964–1974 were only 65% of the average from 1947 to 1963, with the greatest changes occurring in the months of June and July.

Table 1 Change in average annual melting degree day totals

Station	(T_x, T_n, C)		Mean: 1964–76	%*
	First complete annual record	Mean: start of record to 1963		
Thule	1947	937	604†	65
Eureka	1948	808	702	87
Resolute	1948	616	462	75
Alert	1951	505	440	87
Isachsen	1948	458	339	47

*Post-1963 average as a % of pre-1964 average.

†1964–74 inclusive.

How has this change in summer climate affected glacier mass balance in the region? It has been argued that mass balance measurements from the north-west sector of the Devon Ice Cap (taken since 1960 and comprising the longest series of such measurements in the Canadian Arctic) show no evidence of a cooling trend⁶. These data are highly correlated with T_n MDD indices at Resolute and Thule ($r = 0.94$, $y = 2.79x - 224.8$; $P < 0.001$) (Thule and Resolute are the two long-term weather stations closest to the Devon Ice Cap, ~400 km and ~350 km to its east and west, respectively. It is interesting that extrapolation of the relationship to a situation where the annual T_n MDD total is zero results in a mass

balance of 225 kg m⁻² a⁻¹, which is very close to present accumulation amounts on the summit of the ice cap (220 kg m⁻² a⁻¹) (ref. 6.) Using this regression equation, mass balance on the north-west Devon Ice Cap can be reconstructed back to 1947–48, when instrumental observations were first kept at both Thule and Resolute (Fig. 2). By putting the recent mass balance measurements in perspective, the post-1963 change is seen to be highly significant. Between 1947 and 1963, the ice cap continuously lost mass, with greatest losses occurring in the later 1950s. In the last decade, positive balance years have outnumbered negative balance years, although overall there has still been a net mass loss since 1963. This is seen clearly in Fig. 3, where cumulative mass losses since 1947 are shown. Between 1947–48 and 1933–64, we estimate the Devon Ice Cap lost ~3,500 kg m⁻², whereas since 1963–64 the net loss has been <350 kg m⁻². Similar studies using the only other long series of mass balance data in the High Arctic (from the White Glacier, Axel Heiberg Island)^{7,8} show that these results are probably typical of an extensive area of the North American High Arctic and that the change in climate since 1963 has thus had an impact on snow and ice bodies of the region.

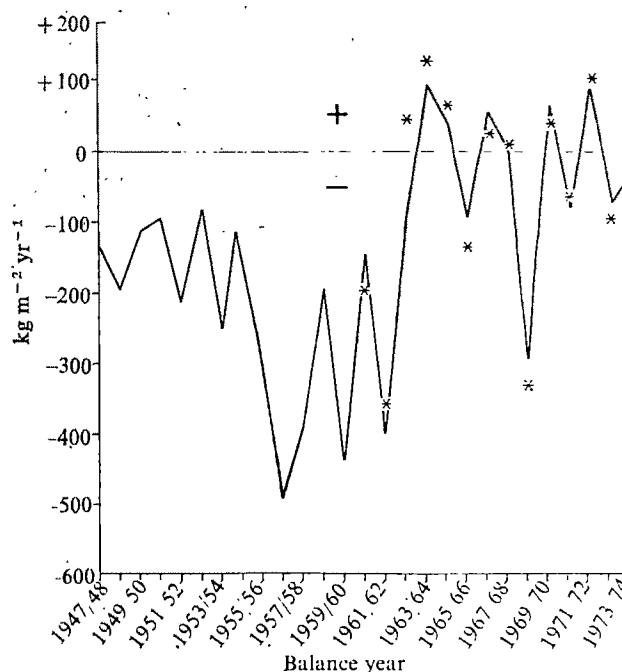


Fig. 2 Reconstruction of mass balance on the northwestern sector of the Devon Ice Cap, based on a regression equation between Devon mass balance since 1960 and average T_n MDD totals at Thule and Resolute. Stars indicate actual mass balance observations from Koerner⁶, 1977.

It is suggested that this change in summer climate is related to the massive input of volcanic dust into the upper atmosphere as a result of the eruption of Mount Agung in March 1963. The total dust veil in the Northern Hemisphere from 1963 to 1968 was the greatest since the period 1883–90, following the huge eruption of Krakatau (6 S, 105 E) in August 1883 (refs 1, 2). Dust veil index values in the mid-1960s were 1,100 compared to 1,500 after Krakatau². Recent work^{10,11} indicates that the greatest change in Northern Hemisphere surface temperatures in the past 25 years was related to the input of Mount Agung dust into the stratosphere. The dust affected solar radiation receipts at high latitudes of the USSR (71–81 °N) by late 1963 (ref. 14), and the effect is clearly seen in solar radiation and temperature data for Resolute (Fig. 4). In summer 1964, diffuse radiation reached the highest levels ever recorded and direct radiation fell to the lowest values on

record. This increase in diffuse and decrease in direct radiation (often with no change in total radiation receipts) is a typical 'volcanic dust signal' and is very similar to that recorded at Aspendale, Australia in summer 1963, when direct radiation was reduced by 24% (by Agung dust in the stratosphere) but diffuse radiation almost doubled¹³. Unfortunately no solar radiation data are available for Resolute before 1961, so it is impossible to compare post-Agung values with any long-term pre-eruption value. However, there is some indication in Fig. 4 that summer diffuse radiation values decreased slowly from 1964 to 1969 (while direct radiation values increased). This may reflect stratospheric dust gradually settling out following the eruption. It is interesting that summer solar radiation receipts in 1972 were similar to 1964; diffuse radiation receipts were very high, and direct radiation very low. The summer of 1972 was the coldest since records began in 1947 (the only summer colder than 1964).

Figure 4 also shows an inverse relationship between summer diffuse radiation and $(T_x + T_n)$ MDD totals at Resolute. The correlation ($r = -0.83$) is statistically significant ($P < 0.01$), and indicates that diffuse radiation totals are closely linked to summer 'warmth', which in turn is a critical factor affecting glacier mass balance, as discussed above. Hence, in periods with large amounts of volcanic dust in the atmosphere, diffuse radiation totals would be high, surface MDD totals would be low and glacier mass balance would be positive.

If the Agung eruption was responsible for the change in ablation season conditions and mass balance during the 1960s, then it is likely that other periods of frequent volcanic activity resulted in temperature changes in the High Arctic at least as large as those observed since 1963. Thus it is probable that in the period 1750–1880 (when there were at least 14 eruptions of a magnitude equal to or greater than that of

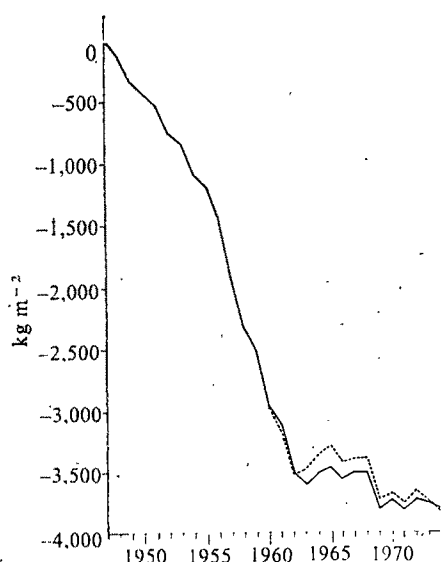


Fig. 3 Reconstruction of cumulative mass loss on the north-west Devon Ice Cap since 1947. The abrupt change in net loss since 1963 is clearly shown.

Agung) ablation season temperatures in the High Arctic were extremely low. Consequently, glacier mass balance was almost certainly positive during this interval, as indicated by stratigraphic studies of the Devon Ice Cap⁸ and the Gilman glacier¹⁴. Conversely, the period 1920–63 (when volcanic activity was exceptionally low) was probably characterised by predominantly warm summers with more negative mass balance conditions¹⁵. Hence, the warmer period seen in Fig. 1 before 1964 was probably typical of summers back to the 1920s and

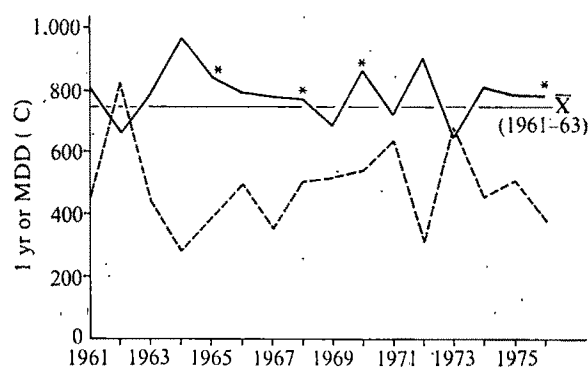


Fig. 4 Mean diffuse solar radiation receipts (June–August) at Resolute, NWT. The marked increase in diffuse radiation following the Agung eruption and the inverse relationship between diffuse radiation and $(T_x + T_n)$ MDD at Resolute ($r = -0.83$) is shown. Solid line, June–August, diffuse radiation; dashed line $(T_x + T_n)$ MDD.

the climate and mass balance conditions of the post-Agung period may be more typical of conditions characteristic of the last century.

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Received 5 December; accepted 30 December 1977.

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Low sea levels, droughts and mammalian extinctions

DRASTIC environmental changes and mammalian extinctions have been reported to coincide with low ocean levels. These low levels were, therefore, interpreted as being caused by major climatic coolings with glacial eustatic regressions, although no continental glaciations, or cooling of Pleistocene-type, has been recorded outside Antarctica earlier than ~ 3 Myr ago. We give here a simple geophysical solution to this problem, which is that any regression (glacial eustatic, tectono eustatic or geoidal eustatic) will also affect the ground water table under the continents by geoid changes and hence lead to drastic environmental and faunal changes, including extinctions. Instead of extinction by temperature decrease, the chain reaction 'regression-drought-extinction' is proposed. Continental geoid fluctuations affecting the ground water table are not only essential for correct interpretation of the pre-Pleistocene climate but also for understanding and modelling of the Pleistocene climate, because the dryness in equatorial and low-latitude regions during ice ages may be interpreted rather as an effect of the lowered geoid

and ground water table than of the temperature decrease, although, in this case, the latter is of a significant order of magnitude.

In documenting periods of drastic environmental changes and mammalian extinctions, two major periods of changes can be recognised¹: one beginning with the Eocene–Oligocene boundary at ~ 37 Myr and one in the late Miocene (Messinian) at ~ 5–6 Myr. Both these periods were found to correspond to major sea-level regressions¹. These changes, however, seem to have been caused by different mechanisms from the Pleistocene ones, which caused major global climatic changes of fairly short duration one of the best examples of the latter being the 10,000 BP change in global climate (the Pleistocene–Holocene boundary), by Behrensmayer¹, now also shown to correspond to a faunal change and a sudden appearance of a microlithic culture in East Africa.

Absolute, 'eustatic', sea-level changes can be brought about in three different ways^{1,2,10}: (1) glacial-eustasy; climatic changes controlling the glacial volume changes and hence the ocean water volume; (2) tectono-eustasy; earth movements (such as orogeny, tectonism, plate tectonics and isostasy) controlling the world ocean basin volume; (3) geoidal-eustasy; gravitational and rotational changes controlling the geoid configuration and ellipsoid.

The first two vertical eustatic type of changes may be regarded as 0-harmonic changes of the geoid (vertical) and the third one as changes in any of the other harmonics (the configuration). None of these changes can occur without corresponding rotational and gravitational changes. A eustatic change in the oceans will, of course, also change the geoid under the continents and by this affect the crustal movements, the ground water table, and so on. This obvious effect of a eustatic regression on the ground water table has not been discussed before, although it seems to be a solution to major Cenozoic changes in local environment and fauna (without having to infer drastic global climatic changes of Pleistocene type) as well as for the dryness in equatorial and low-latitude regions during ice ages³.

Ingle¹ showed that the Oligocene began with a major cooling, sea-level drop and decrease in diversity in several faunal elements. Van Couvering¹ showed that the Eocene–Oligocene transition corresponds to changes from general forests to woodlands both in North America and in Central Asia. Bakker¹ has demonstrated that the Eocene–Oligocene boundary corresponds to a major evolutionary discontinuity during which the top predator sub-guilds were emptied by extinction due to some environmental change initiated by a world-wide regression.

What could have caused the sea-level drop and the corresponding climatic and faunal changes? There are no records of any major glacial increase in Antarctica at this time¹², and if there had been, an Antarctic ice cap increase is not likely to be linked to global climatic changes of the type we know from the Pleistocene.

The regression recorded must have been caused by orogeny and/or plate tectonics increasing the ocean basin volume leading to a tectono-eustatic drop in sea level, with a corresponding lowering of the geoid under the continents. A lowering of the geoid under a continent means lowered ground water table and hence drought and other local environmental changes. This is exactly what van Couvering¹ reported from the Eocene–Oligocene boundary. These environmental changes, in its turn, explain the drastic faunal changes reported by Bakker¹ and Ingle¹. The change in vegetation affects the albedo and may, therefore, lead to some general change in climate. Such an effect may, together with minor glaciers in Antarctica, explain the temperature drop recorded in some ¹⁸O curves from the South Pacific⁴.

The expansion of the Antarctic Ice Cap to the Queen Maud Glaciation⁵, the cooling in the South Pacific^{6,7} and the regression in New Zealand⁸ have been correlated with the regression in the Mediterranean during the Messinian⁹. Although this correlation may be questioned¹⁰ and need further confirmation, there can be little doubt that the Miocene ended with a worldwide glacial-eustatic regression. Ryan¹ presented solid geological–geophysical data on the distinct regression and erosion in the Mediterranean

in relation to the formation of the huge salt layers of the Messinian¹¹. Van Couvering¹ showed that the land vegetation underwent a major change at about 5 Myr (during the Messinian): in North America and in Central Asia, this marks the sudden beginning of prairie formation. Webb¹ showed that the North American vertebrate fauna records a mass extinction somewhere between 7 and 5 Myr without any corresponding increase in number of immigrants, indicating an environmental origin of this extinction. From Florida, he reported a simultaneous regression of at least 40–50 m (followed by a transgression of at least 50 m).

The model suggested for this late Miocene (Messinian) regression–drought–extinction is a very similar pattern to that of the Eocene–Oligocene event, except that this time the regression is caused by glacial-eustasy. No extensive worldwide cooling as in the Pleistocene is suggested, only a chain reaction on the regression: the lowering of the geoid causing a general ground water fall giving rise to drought and vegetational changes that affected the fauna so much that mass extinction is recorded.

The present models offer a simple and logical explanation for the established correlation between regressions, vegetational changes and extinctions during the Cenozoic without having to imply drastic and worldwide changes in climate like those characterising the later part of the Pleistocene (the 'glacial Pleistocene'). The new factor that makes the chain logical is simply the lowering of the geoid under the continents and its effect on the ground water and the drought.

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Fluvially transported charcoal gives erroneous ¹⁴C ages for recent deposits

IN carbon dating of material included in fluvial deposits, it is usually assumed that either there is little age difference between the dateable material and the fluvial deposit, or that the ¹⁴C sample and the deposit in which it is contained are sufficiently old for the age difference to be of little consequence. We report here on how a series of dates on charcoal from the bed of the Macdonald River, New South Wales, Australia, casts doubt on these assumptions and indicates that serious errors can occur in relating the radiocarbon ages of charcoal samples to the fluvial deposits in which they lie.

The Macdonald River drains a catchment area of about 2,000 km², composed almost entirely of flat-lying Triassic sandstones and shales¹. The river flows into Broken Bay (33°55'S, 151°15'E), a large ria drowned during the last rise of sea level². The lower reaches of the Macdonald are tidal, and the valley floor is extensively alluviated. Deposits consist almost entirely of quartz sand with abundant charcoal. Over 95% of the catchment remains forested, mostly by dry sclerophyll woodland, dominated by *Eucalyptus* spp. Bushfires are not uncommon, and were probably an important environmental occurrence long before the arrival of European man less than 200 yr ago³.

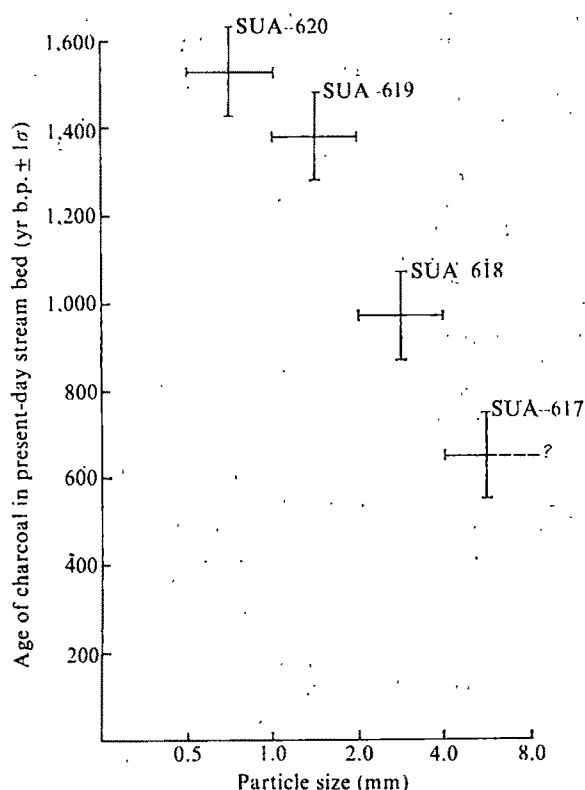


Fig. 1 Radiocarbon age against charcoal particle size.

Sandstone hillslopes in the Macdonald catchment, whence most charcoal fragments must ultimately be derived, provide ample sites where charcoal could be stored. Open joints more than 0.5 m wide and over 1 m deep are filled with loose quartz sand and tree debris; slopewash seems important in sediment transport and fallen sandstone blocks and trees accumulate sediment on their upslope sides; small alluvial/colluvial fans and chutes also store sediment on the slopes which rise as much as 200 m above the river. Although age estimates on the largest trees in the catchment are not available, there seems no reason to doubt that individuals of some *Angophora* and *Eucalyptus* spp. live to ages beyond 200 or 300 yr. Thus some charcoal fragments could have radiocarbon ages of several hundred years before they reach the valley floor.

Sediment storage, transport and redeposition are also characteristic of the alluviated valley floor¹. Channel beds and banks are composed almost entirely of loose sand; bank undercutting and channel migration are common. Even at low flow, discharges of $1 \text{ m}^3 \text{ s}^{-1}$ or less can transport much of the bed load including charcoal fragments. During flood stages extensive alluviation of the floodplain occurs and tabular bars and benches are built on the bed and along the banks. Charcoal is a substantial component of all these fluvial deposits; beds of almost pure charcoal fragments, more than 10 cm thick, and up to several square metres in extent, are quite common in the Macdonald Valley and similar catchments. Given these catchment conditions, there is every expectation that the ages of sediment deposition and the contained charcoal should be at variance.

At a site just upstream of the present tidal limit charcoal fragments were collected from the mobile bed of the present stream during low flow from channel bars only 2–3 cm above water level. The collected charcoal was either in transit or had been mobile in the previous few days. The bulk sample of several thousand grams of wet charcoal was dried and gently sieved into the following size fractions: 0.5–1.0 mm (SUA-620), 1.0–2.0 mm (SUA-619), 2.0–4.0 mm (SUA-618) and > 4.0 mm (SUA-617).

Samples were boiled for 10 min in dilute phosphoric acid, washed with distilled water, soaked overnight at room temperature in an alkaline phosphate solution, washed and boiled again in dilute acid. This pretreatment procedure has been shown to remove acid and alkali soluble contaminants with minimal destruction of the charcoal⁵. Pretreated samples were converted to benzene by standard techniques and counted for an average of 1,200 min each using the liquid scintillation technique⁶.

The radiocarbon ages increase with decreasing charcoal fragment size (Fig. 1), probably as a result of breakdown of the charcoal pieces with time during intermittent transport and storage. Because surface area, and hence the potential for organic contamination, increases with decreasing particle size, the observed trend is the opposite to that expected if contamination were present⁶.

As the sediment with which the Macdonald River charcoal was associated is modern (AD 1976), the relationship between the age of the deposition of the sediment and the age of the charcoal is not simple, but depends on the relative mix of particle sizes in the charcoal sample selected for assay. For example, a sample collected earlier from the same site and assayed without knowledge of its particle size distribution (other than that all fragments were finer than about 8 mm) produced an age estimate of 560 ± 90 yr b.p. (SUA-313).

A similar sample, collected from the present stream bed, but some 26 km upstream from the tidal limit, gave an age estimate of 440 ± 95 (SUA-512/1). A second analysis on a sample from the same plastic bag gave an age estimate of 80 ± 95 (SUA-512/2). The pooled mean of these two results is 260 ± 70 yr b.p. Polach's Z statistic⁷ indicates that the ages of SUA-313 and SUA-512 are significantly different at about the 0.2% level, thus supporting a hypothesis that charcoal fragments associated with present-day fluvial deposits increase in age with distance downvalley. Reasonable as this hypothesis is in the light of current knowledge of floodplain storage of sediment and channel migration⁸, the data presented in Fig. 1 and the lack of charcoal particle size control on SUA-313 and SUA-512 indicate that it cannot yet be substantiated.

In the light of these results, dates on older sediments in the Macdonald River area also present problems of interpretation. A sample of charcoal from a slightly indurated reddish clayey sand exposed in the stream bed near the tidal limit (but believed to be a colluvial deposit) provides an age estimate of $3,350 \pm 110$ yr b.p. (SUA-314). Most of the charcoal pieces in this sample were probably finer than 2 mm.

At a site 10 km upstream, charcoal (finer than about 8 mm) was collected 4.65 m below the surface of a terrace which stands about 7.6 m above the stream bed. This sample was dated at $1,830 \pm 100$ yr b.p. (SUA-315).

Although at the time of sample collection and radiocarbon assay these age determinations were believed to provide reasonably accurate age estimates on the geomorphic surfaces, Fig. 1 indicates that no great significance can be attached to them. We can only assume that the reported ages are maximum ages for the deposits in which the charcoal fragments are enclosed and note that the reported dates and the true ages of the sediments might differ by 1,500 or more years.

There is no reason to believe that the results of assays reported here are unique. Other valleys along the eastern part of New South Wales are very similar in lithology, topography and climate⁹, and we can ask whether similar relationships between charcoal particle size and age do not also apply. Thus, age dates for alluvial fills in the adjacent Wollombi and Colo catchments reported by Hickin and Page⁹ must be regarded as maximum ages, even though these authors showed that the fills were much younger than had previously been supposed. Ages reported by Young¹⁰ and Warner¹¹ for Holocene terrace and floodplain systems along the New South Wales coast must also be treated as maxima that may be seriously in error. We might also surmise that age estimates on estuarine and coastal deposits overestimate the antiquity of landforms where charcoal ulti-

mately derived from terrestrial catchments has been assayed.

It seems unlikely that the longevity of charcoal in fluvial transport systems is peculiar to the eastern New South Wales environment. The results reported here may cast doubt on late Holocene chronologies established in various parts of the world (see ref. 12), and may help to explain the overlapping ages of alluvial fills apparently separated by well-developed soils, or to account for marked differences in age estimates of the one stratigraphic layer where some samples are from hearths, for example, and others are derived from transported charcoal.

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Received 25 October; accepted 15 December 1977.

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Zinc in north-east Pacific water

ALMOST all lead data for the marine environment are inaccurate, contends Patterson¹, because of gross contamination from faulty sampling and analytical procedures. Most marine chemists assume that similar problems are associated with other trace elements as well. Hence, clean sampling and analytical techniques have been adopted. These procedures, in conjunction with the improvement of analytical instrumentation, have resulted in reports on Cu, Ni and Cd (refs 2–4; 3, 5; and 3, 6–8 respectively) levels in seawater that are at least an order of magnitude lower than those previously thought to exist. We report here that Zn concentrations (10–600 ng l⁻¹) are also considerably lower than previously published estimates of 1–30 µg l⁻¹ and that its vertical distribution (surface depletion, deep enrichment) is very similar to that of a major plant nutrient; that is, silicate.

Because Zn is ubiquitous, chances for contamination are at least as great as those for Pb and hence, accurate Zn data can only be obtained using strict precautions. Nevertheless, even with the application of ultra-clean methods, the reliability of Zn data must also be demonstrated by employing at least two independent techniques. Thus, we have been using two different preconcentration procedures (organic extraction and chelex resin exchange) on unfiltered water samples collected with two sampling systems: a modified Go-Flow bottle suspended on Kevlar[®] line and a sophisticated protected-deep-ocean-stable-lead sampler designed and constructed by Schaule and Patterson of the California Institute of Technology. Analyses were performed by flameless atomic absorption spectrophotometry.

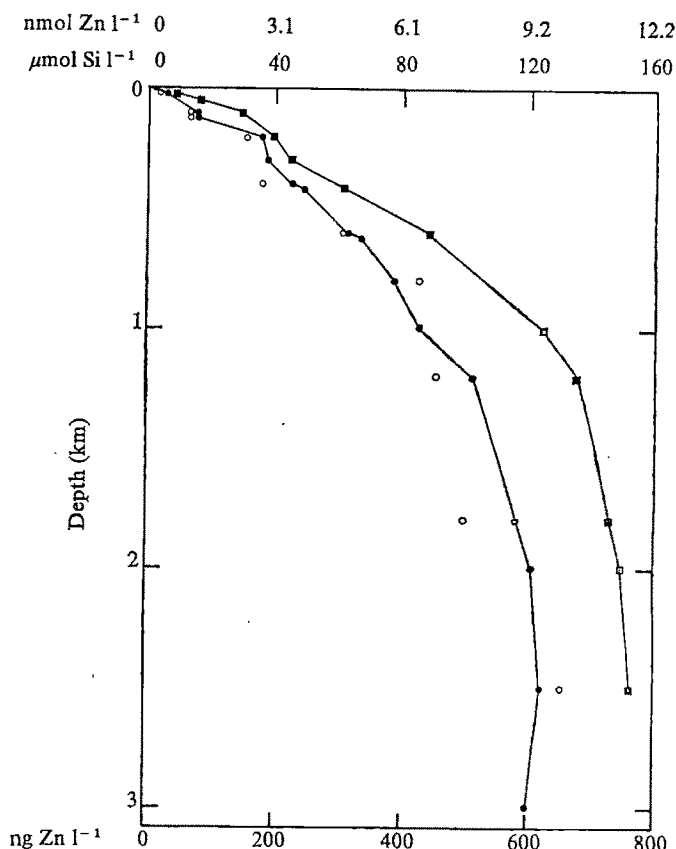


Fig. 1 Depth profiles of Zn and Si (■) off the central California coast (see Table 2). The Zn profile line was drawn using average organic extraction data (●). Chelex values are indicated by (○).

Further details on sampling and analytical methods have been published elsewhere^{7,8}.

The two systems were first compared in December 1976 at a station 100 km west of the central California coast (Table 1). Surface water was collected by holding carefully cleaned bottles below the water surface whilst rowing a raft into clean water. Zinc levels from these samples were then compared with those obtained near the surface using the two sampling systems mentioned above. Replicate analyses using organic extraction were 63 and 38 ng Zn l⁻¹ for the raft samples. These values agreed with those obtained at 30 m using the Go-Flow system (52, 42 ng Zn l⁻¹). Triplicates of the 30 m Go-Flow water also showed similar concentrations (46, 38, 23 ng Zn l⁻¹) when analysed after pre-concentration with chelex. Further evidence that the Go-Flow sampling system did not grossly contaminate samples for Zn was also shown when levels obtained at 920 m with the Go-Flow sampler were compared with those obtained at 1,200 m using the CIT sampler. Replicate analyses using organic extraction at the two depths were remarkably precise and were not significantly different (495, 503 against 519, 515 ng Zn l⁻¹). The 920 m samples analysed using chelex, however, were grossly contaminated. This occurred at some time after the water was withdrawn from the sampling system. (Because of space limitations, chelex pre-concentration was not performed in the filtered-air-clean lab on this cruise.) The remaining Zn values from 1,380 m (Go-Flow) and 2,300 m (CIT) were in close agreement regardless of sampler or preconcentration method. Thus, with the exception of one set of contaminated samples, we believe that the data in Table 1 represent reasonable first estimates for Zn in seawater.

In order to confirm these initial findings and to obtain a more detailed profile, we collected additional samples in the

Table 1 Initial Zn concentrations observed off the central California coast (36° 50'N, 123° 30'W) in December 1976

Depth-sampler (m)	Chelex (ng Zn l ⁻¹)	Organic extraction
0.2 (raft)		63
30 GF	46	38
	38	52
	23	42
920 GF	3,100*	495
	1,030*	503
1,200 CIT		519
		515
1,380 GF	607	549
	590	603
	673	
2,300 CIT		542
		513

Surface water was collected from a raft. The remaining samples were collected using Go-Flow bottles (GF) and Schaule's and Patterson's deep-water-stable-lead sampler (CIT). Zinc was pre-concentrated by organic extraction and chelex resin exchange.

*Obviously contaminated

same general area in April 1977 (Table 2). Essentially the same methods were used, however, on this cruise, all ship-board sample handling was performed in a filtered-air-clean lab and the Go-Flow system was further modified to lessen chance for contamination. Very good agreement was achieved regardless of sampler type or pre-concentration method used. The only exception was the 50 m sample (the first collected) which, we believe, was contaminated. In order to corroborate the precision of the two methods, chelex data were regressed against organic extraction data from the same depths. A correlation coefficient of 0.984 ($n = 11$) was obtained. The resulting equation ($\text{ng organic extract Zn} = 17.3 + 0.99 [\text{chelex Zn}]$) indicated that the organic extraction values were generally higher than those obtained using chelex. The apparent reason for this discrepancy is the fact that minor amounts of Zn (approximately 10 ng l^{-1}) seem to pass through chelex resin. This was observed when selected effluent samples from the chelex columns were analysed by organic extraction. In any event, in terms of both quality and quantity, we believed that the

organic extraction values more closely represented environmental concentrations and these data were used for the depth profile shown in Fig. 1.

At first glance, the depth profile for Zn is not unlike profiles for Cu, Ni and especially Cd (refs 2-4; 3, 5; and 6-8 respectively). Zinc exhibits surface depletion and deep enrichment. What is extraordinary about Zn, however, is the magnitude of the deep enrichment and surface depletion. When the deep maximum value (622 ng l^{-1}) is divided by the average surface raft level (8.5 ng l^{-1}), a ratio of 73 is obtained. In contrast, similar ratios for Cu and Ni are 2-3, while Cd has a ratio of about 30. As our surface Zn values were obtained in California Current waters with appreciable nutrients still present near the surface, it is probable that open ocean surface waters, where nutrients are completely depleted, will have still lower Zn values in the order of $1-5 \text{ ng l}^{-1}$. Hence, deep-surface ratios in the hundreds may also be anticipated.

Phosphate, nitrate and silicate analyses were also performed on many of the water samples collected for Zn. Since maximum PO_4 and NO_3 levels occurred at 800 m, it was immediately apparent that the zinc profile was more nearly similar to that observed for silicate (Fig. 1). A plot of silicate levels against zinc (Fig. 2) yielded a correlation coefficient of 0.992 ($n = 10$). The resulting equation was $\text{ng Zn l}^{-1} = -12.1 + 3.98 (\mu\text{mol Si l}^{-1})$.

This highly significant correlation ($P < 0.01$) between Si and Zn certainly was not expected. Because of zinc's biological involvement with all organisms, one would expect a stronger correlation between Zn and P and N than between it and Si. Clearly, the high correlation between Zn and Si does not necessarily mean that a direct relationship between the two elements actually exists. Nevertheless, Zn's depth profile, the apparent relationship between it and Si, Zn's marked surface depletion and the fact that phytoplankton concentrate large amounts of this element¹⁰ all suggest that diatoms play an important role in the biogeochemical cycling of this element.

We believe that the data reported here are the most accurate yet observed for Zn. Previously reported Zn values ranging from one to tens of $\mu\text{g l}^{-1}$ are undoubtedly the result of faulty sampling and analytical procedures.

We thank B. Schaule and C. C. Patterson of CIT for the aliquots of water provided from their sampler; also Rob Franks of the University of California, Santa Cruz, for

Table 2 Zinc concentrations observed off the central California coast (37° 05'N, 123° 22'W) in April 1977

Depth-sampler (m)	Chelex	Pre-concentration method Organic extraction (ng Zn l ⁻¹)	\bar{x}^* Organic extraction	(nmol Zn l ⁻¹)	$\mu\text{mol Si l}^{-1}$
0.2 (raft)	< 15	7, 10	8	0.13	—
25 GF	18	33, 29	31	0.48	8.60
50 GF	214†	186, 202†	—	—	15.5
100 GF	63	69, 82	76	1.16	29.3
110 CIT	63	76, 74	75	1.15	—
200 GF	149	180, 175	178	2.72	39.7
300 GF	—	186, 192	189	2.89	45.0
400 GF	180	229, 226	228	3.48	—
410 CIT	—	248, 244	246	3.76	—
600 GF	310	314,	314	4.80	88.8
630 CIT	—	322, 352	337	5.16	—
800 GF	428	407, 375	391	5.98	—
		404, 378			
1,020 CIT	—	419, 445	432	6.61	125.0
1,200 GF	456	519, 510	514	7.87	136.0
1,800 GF	500	479, 588	584	8.93	146.2
2,030 CIT	—	641, 576	608	9.31	150.0
2,500 GF	654	628, 617	622	9.52	153.0
2,950 CIT	—	600, 603	602	9.20	—

Surface water was collected from a raft. The remaining samples were collected using Go-Flow bottles (GF) and Schaule's and Patterson's deep-water-stable-lead sampler (CIT). Zinc was pre-concentrated by both organic extraction and chelex resin exchange.

*Mean value

†Contaminated

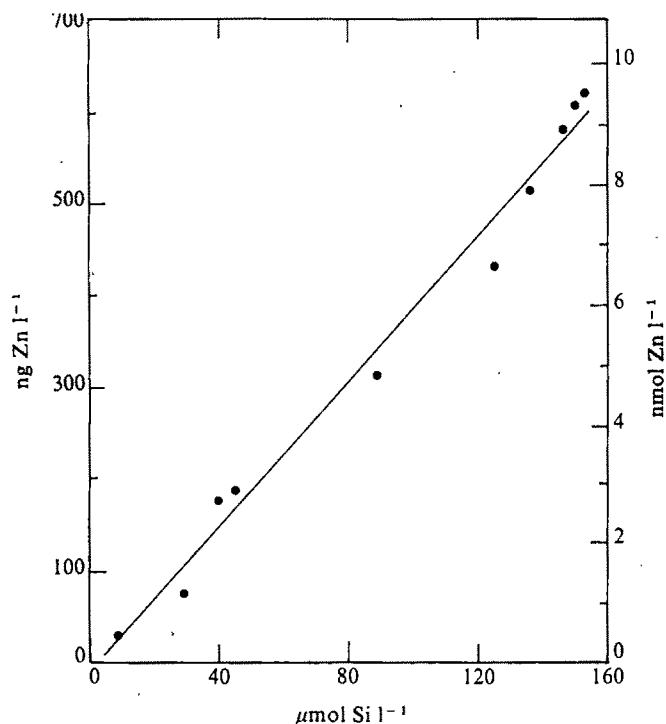


Fig. 2 Average organic extraction Zn values against Si levels measured at the same depths. $r = 0.992$ ($n = 10$): $\text{ng Zn} = -12.1 + 3.98 (\mu\text{mol Si})$; $\text{nmol Zn} = -0.18 + 0.06 (\mu\text{mol Si})$.

his help in developing the sampling and analytical techniques. This research was supported by the US NSF, International Decade of Oceanic Exploration Pollutant Transfer Programme, grant IDO 75-01313 and by the NSF Marine Chemistry Programme, grant OCE 75-13380 and OCE 75-13659.

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Dates, rates and angles of faulting in the Peru-Chile Trench

THE high convergence rate between the Nazca Plate and the South American continent¹ (10 cm yr^{-1}) has been reported to result in rapid rates of vertical tectonism in the trench off northern Peru^{2,3}. This paper presents additional evidence for recent, reverse faulting along the Peru-Chile Trench and new evidence for an older episode of normal faulting. We discuss the implications of this data for the timing and the angles of faulting at convergent plate boundaries.

Steep scarps with offsets of several hundred metres have been described on the outer slope of the Peru-Chile Trench^{2,4,5}. Most of these features are thought to be caused by normal faulting as a result of tensile bending stresses in the upper oceanic lithosphere as the lithosphere descends into the trench^{4,6}. Regional studies show that large fault blocks are not present west of the convergence zone, but begin forming 30-60 km seaward of the trench axis as the oceanic plate begins to bend downward⁴. Using a 10 cm yr^{-1} convergence rate and assuming that the scarps now in the axis formed 30-60 km to the west gives them an age of formation of 300,000 to 600,000 yr BP. This is a maximum age since some faults also form after the plate begins to descend into the trench⁴.

Several of the scarps within the trench axis were dredged during the FDrake cruise in 1975 (Fig. 1). The upper layer 2 basalts recovered⁷ were either freshly fractured with unaltered surfaces or in some instances had thin manganese coatings up to a few tenths of a millimetre thick. None of the basaltic material showed any measurable alteration⁷ of the type reported for basalts exposed to seawater for extended periods of time⁸. Using typical accumulation rates for manganese coatings in the deep ocean of 1 to 4 mm Myr⁻¹ (ref. 9), the thin manganese coatings represent exposure times of a few hundred thousand years or less. Although both methods of estimating the age of faulting are imprecise, they are independent and consistent with each other, suggesting that many of the fault scarps now in the axis formed since 500,000 yr BP.

Continently derived turbidites in two cores displaced high above and seaward of the trench axis have already been used to document vertical movements in the Peru-Chile Trench^{2,3}. Four additional cores have now been sampled and dated by the ¹⁴C method (Fig. 2, Table 1). The cores consist of fine to medium-grained sand turbidites which originate on the continental shelf and slope and flow down into the trench axis

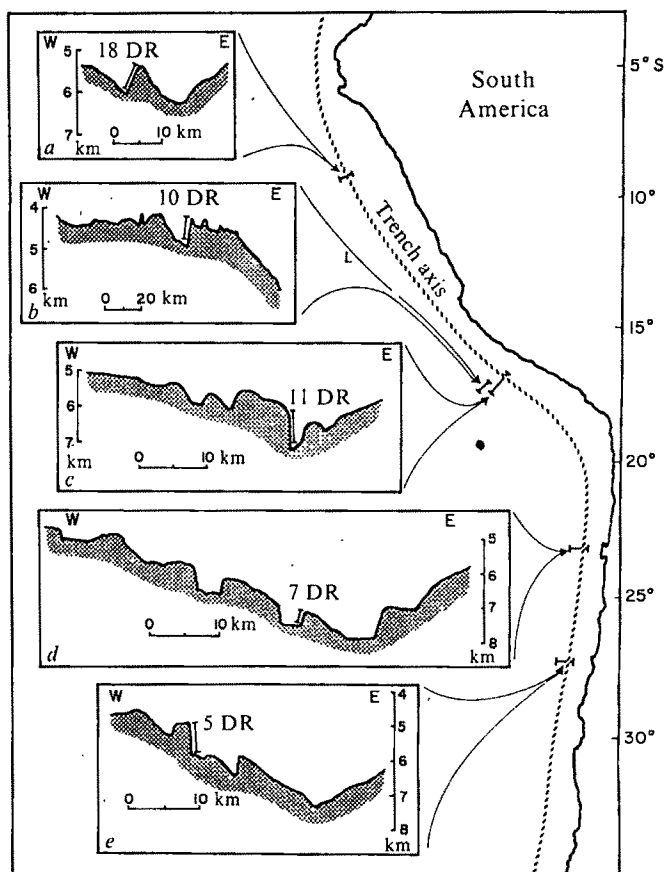


Fig. 1 Dredge sites and fault scarps within the Peru-Chile Trench. Vertical elevation in a, = 9:1; b, = 23:1; c, d and e, = 5:1.

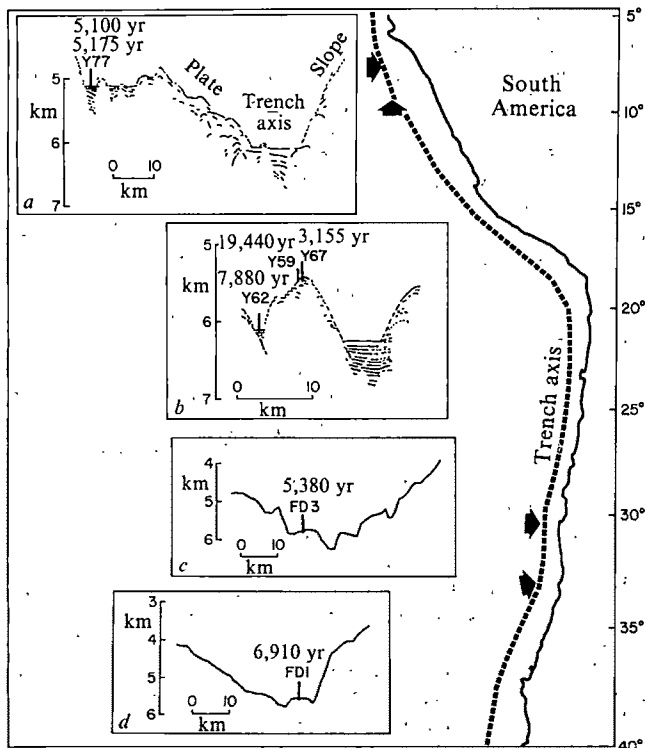


Fig. 2 Core locations and ^{14}C ages of uplifted turbidites in the Peru-Chile Trench. Complete ages and uplift rates listed in Table 1. Vertical elevation in a, = 15:1; b, c and d, = 10:1.

basin². To reach their present elevated positions seaward of the trench axis would require more upslope transport than is achieved by turbidity current flows in any known depositional environment (see, for example ref. 10). Faulting and vertical movement of the oceanic crust within the trench axis is the most feasible mechanism for isolating these deposits above the present level of turbidite deposition. Dating the uppermost turbidite in each core gives a maximum age of initial isolation. Dividing this age by the height above the trench axis yields a minimum rate of vertical displacement (for details see ref. 2, Fig. 10). The calculated rates of displacement for these sites range from 3 to 22 cm yr^{-1} for vertical offsets of up to 990m (Table 1). These rates of vertical tectonism are higher than any others of which we are aware and are to our knowledge the only such estimates made within a trench axis.

Based on regional bathymetry and structures, uplift of small sections of the trench axis seems more probable than a uniform regional downwarping of the areas around the core sites as a mechanism for isolating the turbidites^{2,4}. At the scale of a single fault, uplift can only occur as the result of reverse faulting (maximum compressive stress approximately horizontal and normal to the strike of the fault). Uplift in the trench is thus attributed to horizontal compression from converging, colliding

plates (the dip of the descending oceanic plate is generally 5° or less in the trench and is ignored in our simple model). If all vertical motion is a consequence of horizontal plate convergence and occurs along a single reverse fault (the simplest model), then simple trigonometry gives a minimum dip angle for the fault. By the geometry shown in Fig. 3, the amount of uplift (U), the angle of faulting (α) and amount of convergence (C) are related by: $U = C \tan \alpha$. Using $C = 10 \text{ cm yr}^{-1}$ and U values from Table 1, the calculated minimum angles of reversed faulting range from 16° to 66° (Table 1). Uplift rates and fault angles were calculated assuming continuous fault motion and episodic, represent minimum estimates. If fault movements were rapid and individual pulses of uplift would have been more therefore minimum fault angles would be greater.

Based on the above data, we propose the following model. Normal faults form earliest and are distributed over most of the trench outer slope down to the trench axis, a region approximately 40 km or more wide⁴. With present rates of plate motion, such features will persist a few hundred thousand years before being subducted. Recent reverse faulting in the trench axis and at least a partial decoupling of upper oceanic layer 2 from the

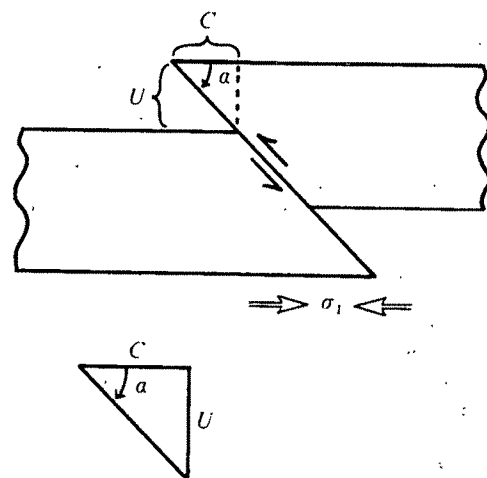


Fig. 3 The relationship between the convergence rate C , uplift rate U and the angle of reverse faulting α . $U = C \tan \alpha$. σ_1 is the axis of maximum compressive stress.

descending oceanic plate², usually along a narrow zone approximately 10 km wide, is indicated by the uplifted turbidites. Because they form in the trench axis, compressional features are relatively short-lived, of the order of 100,000 years or less at the present convergence rate. Further, the areal distribution of normal faults is at least four times that of reverse faults as compressional failure probably does not always extend seaward of the continental slope⁴. Lastly, the larger calculated fault angles (Table 1) support the idea that reversed motion may occur within the trench axis along the older steep normal faults².

Table 1 Turbidite cores: ages, rates and angles of uplift

Core i.d.†	Lat. (s)	Long. (w)	Water depth (m)	Height above axis (m)	^{14}C age (yr b.p.)	Minimum uplift rate (cm yr^{-1})	Minimum fault angle ($^\circ$)
Y77*	8° 10.8'	81° 35.6'	5,121	700	5,100 ± 280	14	54
Y62	9° 23.6'	80° 44.5'	5,936	360	7,880 ± 155	5	25
Y59	9° 20.8'	80° 41.2'	5,304	990	19,440 ± 460	5	27
Y67†	9° 20.5'	80° 41.1'	5,603	700	3,155 ± 145	22	66
FD3	30° 34.4'	72° 37.5'	5,862	340	5,380 ± 350	6	31
FD1	32° 57.3'	72° 42.9'	5,584	200	6,910 ± 230	3	16

*See ref. 3.

†See ref. 2.

i.d., internal diameter

If this model is applied to other trenches, it is apparent why reverse faulting has only just been postulated elsewhere^{11,12}. As actual fault planes are not visible on seismic reflection records, it is impossible to distinguish between a normal fault scarp and a steep reverse fault scarp without independent evidence of the kind provided by uplifted turbidite cores or, as in other detailed studies, by deep tow data and submersible observations^{11,12}.

Finally, the surficial stresses and faults within the trench axis are largely independent of whether the oceanic plate as a whole is under compressional or extensional stress. Even under overall compression flexure of the plate can cause local extensional stress and normal faulting with simultaneous large scale compressional faulting much further seaward of the trench^{1,13}.

We thank L. D. Kulm and K. F. Scheidegger for helpful discussions. Support was provided by the NSF/IDOE Nazca Plate Project.

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Ecdysterone switches off fluid secretion at pupation in insect Malpighian tubules

ECDYSTERONE has been shown to stimulate fluid secretion in Malpighian tubules of adult male tsetse flies, suggesting a new function for steroid hormones in the control of insect excretory systems¹. I report here that ecdysterone has the reverse effect on larval tubules and switches off fluid secretion just before pupation.

Malpighian tubule fluid secretion was measured in larval and pupal male skipper butterflies (*Calpodethlius*, Lepidoptera: Hesperidae). Fluid secretion increases during the last larval stage to 2.02 nl min⁻¹ mm⁻¹ at P-48 (48 h before pupal ecdysis) but is reduced to zero by P-21 (Fig. 1) where it remains until halfway through the pupal stage. Fluid transport also stops at pupation in the Malpighian tubules of the monarch butterfly *Danaus* and the mealworm *Tenebrio* (Table 1), suggesting that it is a general phenomenon among holometabolous insects.

The titre of ecdysterone has been shown to increase in the blood before pupation in several insects²⁻⁴, including *Calpodethlius* (R. Dean *et al.*, personal communication). This suggested that ecdysterone may control the cessation of fluid transport. When ecdysterone is injected into a P-72 stage larva, fluid secretion is switched off within 24 h (Fig. 2). The direct action of ecdysterone on the Malpighian tubules was demonstrated by culturing tubules from a P-72 stage larva *in vitro* with or without 1 µg ml⁻¹ ecdysterone (2 × 10⁻⁶ M) for up to 48 h and then measuring the rate of fluid secretion in artificial haemolymph. As shown in Fig. 3, ecdysterone

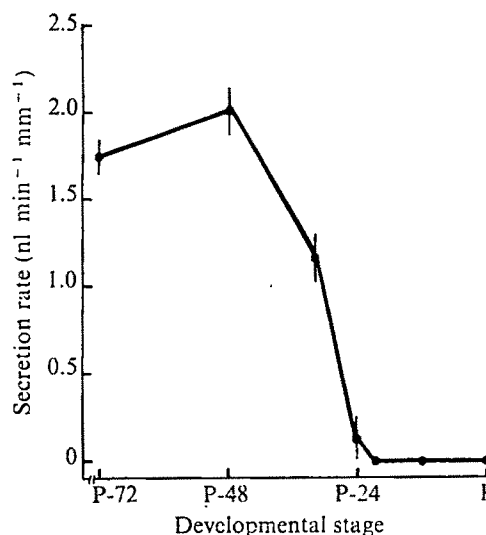
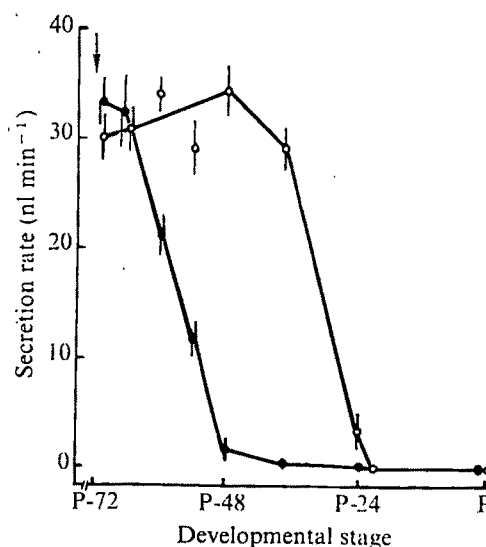


Fig. 1 *In vitro* Malpighian tubule secretion rates at 0, 12, 21, 24, 33, 48 and 72 h before pupal ecdysis (P) in *Calpodethlius*. Secretion rates were measured on isolated tubules in artificial haemolymph (g l⁻¹: KCl 1.87, Na₂HPO₄ 0.71, MgCl₂ · 6H₂O 2.03, CaCl₂ 0.44, glucose 8.98, alanine 0.5, glutamic acid 0.37, glycine 2.26, histidine 2.03, lysine 1.04, serine 1.9, threonine 0.26, streptomycin sulphate 0.3, penicillin 0.03, phenol red 0.006) following the technique of Ramsay⁵. Each point is the average of about 20 tubules ± 1 s.e.m.

caused a significant reduction in fluid transport relative to controls within 12 h, and after 48 h, 13 of 19 experimental tubules were completely switched off.

Fluid secretion depends on energy from mitochondria⁵ and the diffusion of water along ionic concentration gradients in intracellular and extracellular channels^{6,7}; tubule ultrastructure was therefore examined to determine if changes in mitochondrial distribution or in apical/basal channel geometry were associated with the cessation of fluid transport. Electron microscopy revealed that the cells are extensively remodelled during metamorphosis, although the tubules remain intact and persist into the adult stage. Retraction of elongate mitochondria from the apical microvilli,

Fig. 2 Ecdysterone switches off fluid secretion within 24 h *in vivo*. 5 µg ecdysterone in 5 µl 100% ethanol (●) or 5 µl 100% ethanol (○) were injected into P-72 stage larvae (arrow). Secretion rates were subsequently measured using an *in situ* Malpighian tubule preparation in which decapitated and ligated animals were immersed in artificial haemolymph and dissected so that the tubules were left intact distally to the cryptonephridium. 8-18 tubules per point ± 1 s.e.m.



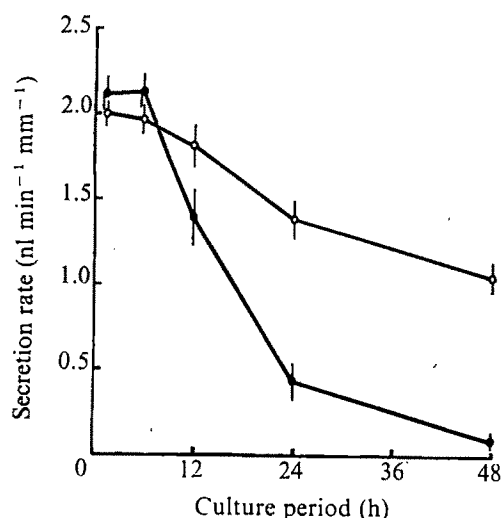


Fig. 3 Ecdysterone switches off Malpighian tubule fluid secretion *in vitro*. Tubules from a P-72 stage larva were cultured in a 50 : 50 mixture of artificial haemolymph-Graces culture medium containing $1 \mu\text{g ml}^{-1}$ ecdysterone (●) or $1 \mu\text{l ml}^{-1}$ ethanol (○). After various periods of time the tubules were transferred to artificial haemolymph and the rate of secretion was measured. Each point is the average of 15–20 tubules ± 1 s.e.m.

with their isolation and degradation in autophagic vacuoles, and a reduction in apical and basal channels are early events in the remodelling process. Both of these events are induced by ecdysterone *in vitro* (ref. 8 and unpublished data).

Fluid transport continues in Malpighian tubules at larval-larval moults (my unpublished data), even though a pulse of ecdysterone occurs in the blood to trigger moulting. This suggests that the inhibitory effect of ecdysterone is

Table 1 Loss of fluid secretion at pupation in different insects

Insect	Secretion rate in last larval stage			Secretion rate in early pupal stage		
		s.e.m.	n		s.e.m.	n
<i>Calpodes ethlius</i>	31.0 nl min^{-1}	1.2	20	0 nl min^{-1}	0	18
<i>Danaus plexippus</i>	5.7 nl min^{-1}	0.6	12	0 nl min^{-1}	0	12
<i>Tenebrio molitor</i>	0.25 nl min^{-1}	0.03	15	0 nl min^{-1}	0	15

modulated by some other factor such as juvenile hormone, since topical application of juvenile hormone to last larval stage *Calpodes* prevented the tubules being switched off at the larval-pupal moult.

Ecdysterone stimulates fluid secretion in tsetse fly tubules within minutes, suggesting a short-term physiological regulation¹. In contrast, the functional and structural changes described in this report are longer-term developmental processes which require hours or days. Also, unlike tsetse fly tubules, no short-term stimulation or inhibition of fluid secretion was observed within the first few minutes or hours following applications of $1 \mu\text{g ml}^{-1}$ ecdysterone to larval or adult *Calpodes* Malpighian tubules.

I thank Professor M. Locke for reading the manuscript and the NRC of Canada for financial support.

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Received 17 October; accepted 10 December 1977.

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Difficulties in auditory organisation as a possible cause of reading backwardness

LEARNING to read and write involves auditory perception, for the child must learn how different kinds of sounds are written. It might seem, however, that although auditory perception is essential to reading, it would not be a significant source of difficulty, for, apart from a few exceptional cases, most children who have difficulties with reading can hear perfectly well, and can discriminate and understand the words which they signally fail to read¹. But discriminating words is not the only aspect of audition involved in reading. The child must also be able to group together words which are different but which have sounds in common. If he is to learn the rules of reading and writing he must understand that 'hat', 'cat' and 'mat', though different, nevertheless have a sound in common. We report here results which suggest that difficulties in this kind of grouping may be a significant source of difficulty in learning to read.

We compared a large group of children of normal intelligence, but 18 months or more behind the average reading skill for their age, with a group of younger children also of normal intelligence, whose reading skills were normal for their age and were the same as those of the backward readers. Details of the two groups are given in Table 1. Although the two groups were approximately equal in reading ability and were both of normal intelligence for their age, the backward readers were on average over three years older than the other group.

This is a novel kind of comparison and our reason for making it was to distinguish between cause and effect. The vast majority of studies of reading backwardness² and all the studies of auditory perception in backward readers³ compare backward with normal readers of the same age and intellectual level, the only difference between the groups being in how far they have learned to read. The trouble with this traditional design is that any difference which is found between backward and normal readers might just as well be the result of the former group's limited experience in reading. But if, as in our design, the two groups have reached the same reading level, and yet the backward readers are worse on a perceptual task, the fact that the two groups have the same reading ability as one another rules out the possibility that the backward readers' perceptual failure is merely the result of a lack of reading experience.

The method which we used in experiment 1 to test the grouping of sounds was to say four monosyllabic words to them. Three of the words had a sound in common which the fourth did not share. The child had to say which was the odd word out. There were three series, each with six trials (18 trials in all). In one series, all four words always had the same middle phoneme, but the last two phonemes were the same in three of the words while the odd one word had a different final phoneme (for example, weed, peel, need, deed). Another series was the same except that the middle phoneme was different in the odd word (for example, nod, red, fed, bed). In the third series, three words had the same opening phoneme while the odd one did not (for example, sun, see, sock, rag). The position of the odd word varied systematically in all three series.

We ensured that all the children understood and could perform the oddity task in practice trials, and we also eliminated forgetting words as a cause of failure, by preliminary trials in which children were given four words at a time and asked to recall them. We discarded two backward readers who consistently failed in these trials; all the others made virtually no memory errors. We took great care to pronounce each word with the same emphasis in order not to give the child any additional cue to the correct word. The experimenter also always hid her mouth from the child's view with a card, so that

Table 1 Details of the two groups

	N	Age		IQ (WISC)		Reading age (Neale)		Spelling age (Schonell)	
		Mean	Range	Mean	Range	Mean	Range	Mean	Range
Backward readers	60	10 yr 4 mth	8 yr 4 mth–13 yr 5 mth	108.7	93–137	7 yr 7 mth	6 yr–9 yr 4 mth	6 yr 10 mth	5 yr–8 yr 9 mth
Normal readers	30	6 yr 10 mth	5 yr 8 mth–8 yr 7 mth	107.9	93–119	7 yr 6 mth	6 yr–9 yr 2 mth	7 yr 2 mth	5 yr 1 mth–10 yr 2 mth

the shape of her mouth would not provide any additional cue for any of the children.

This experiment (Table 2) produced a startling difference between the two groups, the backward readers being markedly worse than the normal group in all three series. Putting the series together, 91.66% of the 60 backward readers made errors and 85% made more than one error. Only 53.33% of the 30 normal readers made any errors and only 26.66% more than

difference between backward and normal readers in categorising sounds was not due to the fact that we sometimes unconsciously emphasised one word more than another, despite our attempts not to do so. This evidence came from experiment 2, with the same children. They were given 10 words spoken successively (dish, car, boat, train, ball, mouse, dog, rake, truck, tent), and asked each time to produce a word which rhymed with each of these words. Here no extraneous cues of emphasis could possibly provide the correct answer.

Again, despite their superior age and overall intellectual ability, the backward readers were by far the worse of the two groups (Table 4), 38.33% of the former group and only 6.66%

Table 2 Mean error scores (out of 6) in experiment 1

Series	Odd word	Backward readers		Normal readers	
		N	s.d.	N	s.d.
1	Last letter different	60	1.15	30	0.17
2	Middle letter different	60	1.43	30	1.11
3	First letter different	60	1.49	30	0.37
		60	2.62	30	0.99
		60	2.26	30	0.67
		60		30	1.188

one. This difference is all the more remarkable, given that the backward reading group, being older by an average of 3½ years, was actually of a considerably higher intellectual level than the normal reading group. We suggest that many backward readers may be held back by a particular difficulty with organising sounds.

Although the backward readers were worse on all three series ($F: 32.499$; $d.f. 1,88$; $P < 0.001$ in an analysis of variance), they were at a particular disadvantage to the normal readers with

Table 4 Number in each group producing failures in experiment 2

	Total N	No. of failures									
		0	1	2	3	4	5	6	7	8	9
Backward readers	60	37	4	4	4	2	2	2	2	0	0
Normal readers	30	28	1	0	0	0	1	0	0	0	0

of the latter failing to produce a rhyming word in one or more trials. This task was probably easier than the earlier oddity test, since in both groups more children succeeded on every trial. But the relative failure of the backward readers in the second experiment is striking confirmation of their difficulty with categorising sounds. Overall, our results strongly suggest that this difficulty could be an important cause of reading failure.

Table 3 Division of the two groups into those making one or no errors and those making more than one error in experiment 1

N	Backward readers		t test of the difference	Normal readers		t test of the difference
	One or no errors	More than one error		One or no errors	More than one error	
	9	51		22	8	
Mean age	10 yr 6 mth	10 yr 3 mth	0.59	7 yr 1 mth	6 yr 4 mth	2.25*
Mean IQ	112.55	108.06	NS	109.73	102.87	2.63*
Mean reading age	7 yr 11 mth	7 yr 6 mth	1.51	7 yr 9 mth	6 yr 8 mth	2.91†
Mean spelling age	7 yr 4 mth	6 yr 9 mth	2.05*	7 yr 6 mth	6 yr 4 mth	2.41*

* $P < 0.05$

† $P < 0.01$

the series in which three of the four words had the same opening phoneme ($F: 4.28$; $d.f. 2,176$; $P < 0.05$). The relationship of this difficulty with the first phoneme to these children's problems with reading and writing should be investigated.

The large size of our groups enabled us to distinguish between those children who made more than one error over the three series and those who made only one error or none at all (Table 3). A clear developmental trend was found among the normal readers, as those who made one or no errors were significantly older and had significantly higher intelligence quotient (IQ) scores, and reading and spelling ages. However no such trend was found among the backward readers; here the only significant difference was that the few children who made one or no errors had a significantly higher spelling age than the rest. This suggests that difficulties in organising sounds may have particularly harmful effects on spelling among backward readers.

We needed further evidence to demonstrate that the large

We thank the Oxford Area Health Authority, the Human Development Research Unit at the Park Hospital, and the SSRC for support for this research, and the Oxfordshire, Berkshire, and Buckinghamshire Education Authorities for their cooperation.

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Received 22 September; accepted 30 December 1977.

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Effect of gene dosage on expression of mitochondrial malic enzyme activity in the mouse

THE region of chromosome 7 in *Mus musculus* in which the albino (*c*) locus is located is of special interest because it is involved in deficiencies¹⁻⁶, a duplication^{7,8}, and X-autosome translocations⁹⁻¹¹. The structural gene (*Mod-2*) for mitochondrial malic enzyme (MOD-2) maps about 1 centimorgan (ref. 12) to the distal side of *c*, and has been shown by genetic experiments^{4,5} to be deleted in certain lethal albino mutations. Over 30 independently isolated homozygous lethal *c*-locus mutations have been recovered and characterised at Oak Ridge. In this report we have compared the level of MOD-2 expression in animals heterozygous for lethal *c*-locus mutations (*c^{ch}/c**) with that of their *c^{ch}/c^{ch}* littermates in order to clarify the precise

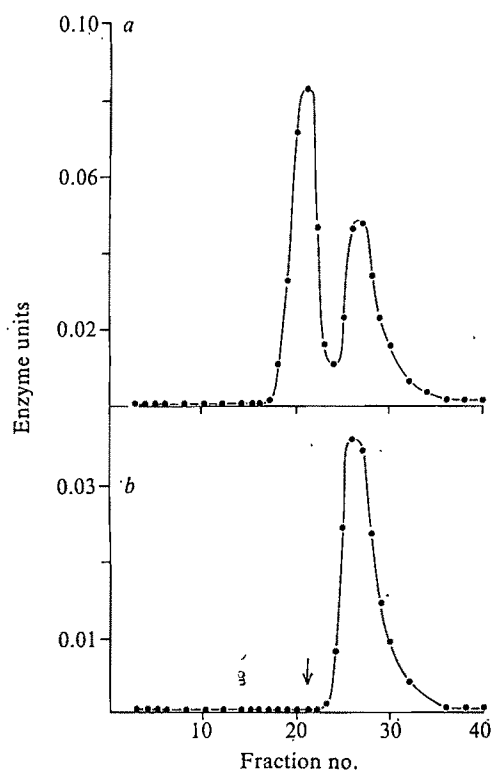


Fig. 1 Elution of heart malic enzymes from DEAE-cellulose. *a*, Total heart; *b*, heart mitochondria. Hearts from BLH mice were homogenised in HB:0.25 M sucrose, 0.05 M Tris-HCl (pH 7.5), 0.025 M KCl, 0.005 M MgCl₂, 100 µg ml⁻¹ heparin and 0.001 M phenylmethylsulphonylfluoride (PMSF). The homogenate was centrifuged for 10 min at 500g. In *a*, this supernatant was brought to 1% Triton X-100 and dialysed overnight against buffer A:0.1 M Tris-HCl (pH 8), 20% glycerol, 0.001 M MgCl₂, 0.001 M PMSF, 0.001 M dithiothreitol and 0.1% Triton X-100. The dialysed extract was centrifuged for 30 min at 30,000g and the supernatant was applied to a column (1 × 15 cm) of DE-52 cellulose (Whatman) that had been equilibrated and washed with buffer A. Elution was carried out with a linear gradient of KCl (0–0.2 M) in buffer A. Fractions of 5 ml were collected from which 0.2-ml aliquots were assayed for malic enzyme activity in a reaction mix containing 0.04 M triethanolamine-HCl (pH 7.5), 0.004 M MnCl₂, 0.00023 M NADP and 0.001 M K-malate (pH 7.6). The reduction of NADP was monitored by the increase in A₃₄₀ on a recording spectrophotometer maintained at 26°C. One unit of malic enzyme reduces 1 µmol NADP per min. In *b*, a mitochondrial pellet was prepared from the 500g supernatant by centrifugation for 5 min at 7,700g. The pellet was then washed twice with HB by centrifugation and was lysed in buffer A containing 1% Triton X-100. Chromatography on DEAE-cellulose was carried out as above after dialysis against buffer A and centrifugation. The arrow indicates the expected elution position of the first peak.

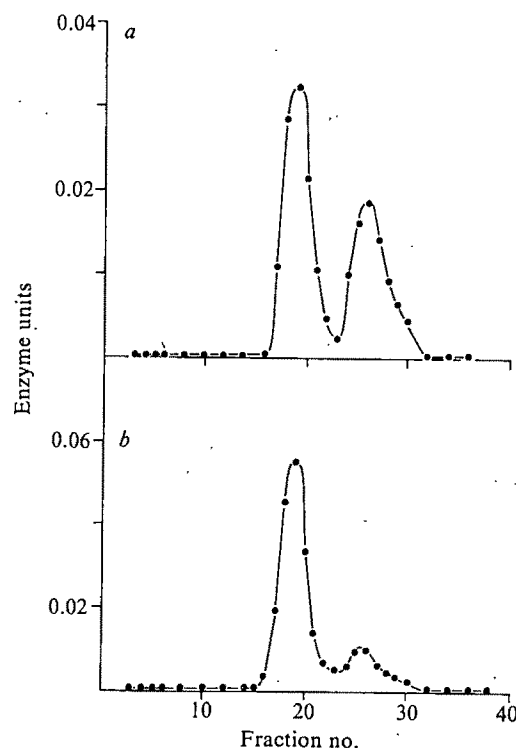


Fig. 2 Elution from DEAE-cellulose of heart malic enzymes from *c^{ch}/c^{ch}* mice (*a*) and from *c^{ch}/c^{ch}** littermates (*b*). Extracts were prepared from *c^{ch}/c^{ch}* and *c^{ch}/c^{ch}19DTR* mice as described in Fig. 1*a*. Chromatography and enzyme assays were carried out as in Fig. 1.

quantitative effect of gene dosage on the expression of enzyme activity in this system. We have shown that enzyme activity is linearly related to gene dosage in both heart and kidney. The data demonstrate that four out of seven complementation groups of *c** mutations are most simply explained as deficiencies that include *Mod-2*.

The specific activity of MOD-2 was determined in mitochondrial pellets prepared from homogenates of whole hearts. For purposes of dosage measurements it was essential to determine what proportion of malic enzyme activity in the mitochondrial fraction was MOD-2. Figure 1*a* shows the gradient elution profile from DEAE-cellulose of total heart malic enzyme, whereas Fig. 1*b* shows the elution of mitochondrial malic enzyme. It is clear that only the later-eluting of the two distinct peaks in Fig. 1*a* was associated with the mitochondrial fraction. Thus, our assays measured only a mitochondrial form of malic enzyme. Evidence that this second peak was in fact MOD-2 is shown in Fig. 2. In this experiment, whole heart homogenates were prepared from *c^{ch}/c^{ch}* and *c^{ch}/c^{ch}19DTR* mice, which were then chromatographed on DEAE-cellulose. The specific activity of mitochondrial malic enzyme in the *c^{ch}/c^{ch}19DTR* heterozygotes is about half that in *c^{ch}/c^{ch}* littermates (see Table 1). The striking decrease in peak II activity in heterozygotes is evident in Fig. 2*b*. We concluded that valid quantitative comparisons of MOD-2 activity could be made between mitochondrial fractions isolated from the hearts of *c^{ch}/c^{ch}* and *c^{ch}/c^{ch}** mice.

Table 1 presents the results of determinations of MOD-2-specific activities in heart mitochondrial fractions of *c^{ch}/c^{ch}* and *c^{ch}/c^{ch}** mice from 32 independently isolated *c** mutant stocks comprising seven complementation groups. These groups clearly fall into one of two classes: those in which heterozygotes were found to have almost exactly half the specific activity of MOD-2 of their *c^{ch}/c^{ch}* littermates, or those in which MOD-2 expression in heterozygotes was indistinguishable from that found in *c^{ch}/c^{ch}* littermates. The former class (groups B, D, E and F) is likely to comprise deletions extending into or beyond the *Mod-2* locus,

whereas the latter class (groups A, A' and C) maintains a functional *Mod-2* locus. Statistical analysis showed highly significant differences between MOD-2 specific activities of c^{ch}/c^{ch} and c^{ch}/c^* animals in every case in groups B, D, E and F; whereas ratios obtained in classes A, A' and C were in no case significantly different from unity.

Table 2 shows the results of three experiments that examined the relationship between *Mod-2* gene dosage and the expression of MOD-2 activity in kidneys. Two stocks previously shown to have a 2:1 ratio of MOD-2 activity in the hearts of c^{ch}/c^{ch} versus c^{ch}/c^* littermates were used. Although insufficient numbers of animals were examined for purposes of statistical analysis, the proximity of the experimental results to the expected 2:1 ratio is evident.

The results of this study have demonstrated that the level of MOD-2 activity is directly proportional to the number of copies of *Mod-2* present in the deletion mutants examined. The use of purified mitochondrial fractions permitted the direct measurement of MOD-2 activity without a large background, of uncertain magnitude, of MOD-1 activity¹³. A series of measurements

made on mice carrying three copies of *Mod-2*, due to a duplication of part of chromosome 7 (refs 7 and 8), has shown that MOD-2 activity is linearly related to gene dosage when two or three copies of *Mod-2* are present (E.G.B. and L.B.R., unpublished). Experiments are in progress to measure MOD-2 activity in mice carrying one, two and three copies of *Mod-2* as progeny of one genetic cross [$Dp(c^{ch})/c \times c^{ch}/Df(c)$]. In a detailed study of the Oak Ridge *c*-lethal mutations, DeHamer⁶ was able to detect clear differences in the staining intensity of the MOD-2 band on starch gels from c^{ch}/c^* and c^{ch}/c^{ch} animals in 23 of the 30 stocks examined, indicating that the *Mod-2* locus was deleted in these mutants. No differences in MOD-2 activity were noted on gels that were run of extracts prepared from c^{ch}/c^{ch} and c^{ch}/c animals (where *c* is a viable albino mutation and, presumably, not a deletion). Quantitative data on the expression of MOD-2 on gels were not obtained.

Our quantitative determinations were totally in agreement with the starch gel patterns obtained by DeHamer⁵, whose results were used in confirming the deficiency nature of most of the *c*-lethal mutations¹ in orientating the complementation map

Table 1 Specific activity of MOD-2 in heart mitochondria of c^{ch}/c^{ch} and c^{ch}/c^* mice

Stock	No. of pairs assayed*	Specific activity† \pm s.e.m.		Ratio \pm s.e.m.
		c^{ch}/c^{ch}	c^{ch}/c^*	$c^{ch}/c^{ch} : c^{ch}/c^*$
Complementation group A				
14CoS	4	0.0161 \pm 0.0009	0.0160 \pm 0.0009	1.01 \pm 0.08
16HATH	3	0.0128 \pm 0.0003	0.0131 \pm 0.0014	0.97 \pm 0.11
15R60L	4	0.0124 \pm 0.0003	0.0134 \pm 0.0003	0.92 \pm 0.03
10R75M	4	0.0110 \pm 0.0007	0.0108 \pm 0.0003	1.02 \pm 0.07
1FAFyh	4	0.0126 \pm 0.0007	0.0109 \pm 0.0002	1.15 \pm 0.07
			Average ratio (A):	1.01 \pm 0.03
Complementation group A'				
3YPSp	4	0.0130 \pm 0.0006	0.0135 \pm 0.0009	0.96 \pm 0.08
23DVT	4	0.0098 \pm 0.0012	0.0106 \pm 0.0005	0.93 \pm 0.13
1DThW _b	4	0.0117 \pm 0.0004	0.0109 \pm 0.0008	1.07 \pm 0.09
			Average ratio (A'):	0.99 \pm 0.06
Complementation group B				
4PB	4	0.0151 \pm 0.0005	0.0074 \pm 0.0003	2.05 \pm 0.11
11DSD	4	0.0101 \pm 0.0011	0.0048 \pm 0.0007	2.12 \pm 0.38
3R60L	4	0.0127 \pm 0.0010	0.0071 \pm 0.0002	1.79 \pm 0.16
2R145L	4	0.0118 \pm 0.0007	0.0060 \pm 0.0004	1.98 \pm 0.18
3R145L	4	0.0118 \pm 0.0008	0.0065 \pm 0.0003	1.82 \pm 0.14
1FDFoHr _c	5	0.0170 \pm 0.0009	0.0087 \pm 0.0006	1.96 \pm 0.16
1FR60H _b	4	0.0147 \pm 0.0005	0.0069 \pm 0.0001	2.12 \pm 0.07
9FR60H _b	4	0.0139 \pm 0.0005	0.0068 \pm 0.0004	2.04 \pm 0.13
14FR60H _b	4	0.0122 \pm 0.0004	0.0065 \pm 0.0003	1.87 \pm 0.11
4FR60H _d	4	0.0156 \pm 0.0012	0.0068 \pm 0.0002	2.28 \pm 0.18
5FR60H _g	3	0.0168 \pm 0.0002	0.0079 \pm 0.0010	2.12 \pm 0.27
			Average ratio (B):	2.01 \pm 0.06
Complementation group C				
20FATw	4	0.0097 \pm 0.0004	0.0102 \pm 0.0005	0.95 \pm 0.06
Complementation group D				
146G	5	0.0135 \pm 0.0004	0.0060 \pm 0.0003	2.26 \pm 0.13
39SAS	8	0.0151 \pm 0.0007	0.0065 \pm 0.0003	2.34 \pm 0.16
19DTR	4	0.0179 \pm 0.0012	0.0078 \pm 0.0005	2.29 \pm 0.22
24R145L	4	0.0132 \pm 0.0003	0.0064 \pm 0.0002	2.05 \pm 0.08
7R250H	4	0.0134 \pm 0.0004	0.0065 \pm 0.0001	2.07 \pm 0.08
68G	4	0.0130 \pm 0.0003	0.0058 \pm 0.0003	2.25 \pm 0.15
202G	7	0.0146 \pm 0.0013	0.0062 \pm 0.0007	2.33 \pm 0.33
10FR60L	1	0.0127	0.0063	1.94
			Average ratio (D):	2.19 \pm 0.06
Complementation group E				
65K‡	2	0.0130 \pm 0.0006	0.0060 \pm 0.0005	2.16 \pm 0.20
112K‡	4	0.0150 \pm 0.0013	0.0064 \pm 0.0005	2.33 \pm 0.26
			Average ratio (E):	2.25 \pm 0.16
Complementation group F				
26DVT	4	0.0132 \pm 0.0004	0.0064 \pm 0.0004	2.05 \pm 0.14
12FR60H _b	3	0.0135 \pm 0.0006	0.0072 \pm 0.0005	1.87 \pm 0.16
			Average ratio (F):	1.96 \pm 0.11

For these experiments, pairs of littermates of like sex were used; that is, activities were determined for a set of c^{ch}/c^{ch} animals, each of which had a littermate of like sex, but of genotype c^{ch}/c^ , whose activity was measured in the same experiment. All determinations on a given stock were made in one day, except for c^{ch}/c^{ch} , 202G, 112K, 1FDFoHr_c, and 146G. Two of the four pairs assayed in stock 3R60L were not littermates, but were of like sex.

†Specific activities are given as units per mg protein. The unit is defined in Figure 1, in which the assay is also described. Mitochondrial pellets were lysed on ice in 0.05 M Tris-HCl (pH 7.5), 0.025 M KCl, 0.005 M MgCl₂, 0.1% 2-mercaptoethanol, and 1% Triton X-100, then centrifuged at 10,000g for 10 min. The supernatant was assayed for enzyme activity and protein. Protein was measured by the method of Lowry *et al.*¹¹.

‡65K and 112K occurred as a cluster, presumably derived from a single irradiated spermatogonium, and are thus not independent mutations.

with respect to the genetic map² and in defining complementation group E (ref. 2). In contrast to the preliminary complementation map presented by Gluecksohn-Waelsch *et al.*⁶, the deficiency in stocks *c^{85K}* and *c^{112K}* (group E) does in fact include *Mod-2* (Table 1 and ref. 5).

Russell and DeHamer reported that progeny of B × E crosses are viable although not fully vigorous, and, on the basis of the starch gel results, concluded that MOD-2 activity was not required for survival. The present results, which indicate that there is only one copy of MOD-2 both in *c^{ch}/c^{B group}* and in *c^{ch}/c^{B group}* animals, indicate that, in fact, the viable *c^{B group}*/*c^{E group}* mice lack MOD-2 altogether. We were unable to detect any activity in mitochondrial pellets prepared from *c/c* progeny of a B × E cross, thus confirming this inference.

Table 2 Specific activity of MOD-2 in kidney mitochondria of *c^{ch}/c^{ch}* and *c^{ch}/c^{*}* animals

Stock	Specific activity		Ratio
	<i>c^{ch}/c^{ch}</i>	<i>c^{ch}/c[*]</i>	<i>c^{ch}/c^{ch}</i> : <i>c^{ch}/c[*]</i>
4PB (experiment 1)*	0.0095	0.0056	1.70
4PB (experiment 2)*	0.0097	0.0048	2.02
202G†	0.0170	0.0095	1.80

Kidney mitochondria were prepared as described in Fig. 1, and assays were carried out as described in Table 1.

*Kidneys from three animals of each class were pooled.

†Kidneys from four animals of each class were pooled.

Diamond and Erickson¹³ were unable to detect any significant difference in MOD-2 specific activity in kidney mitochondria from *c^{ch}/+* and *c^{*}/+* animals. The results shown in Table 2 indicate that the expected quantitative difference between *c^{ch}/c^{ch}* and *c^{ch}/c^{*}* mice is found in kidney as well as in heart. We have noted that, in contrast to results with heart mitochondrial fractions, about 20% of malic enzyme activity in extracts of crude mitochondrial pellets from kidney chromatographs in the position of MOD-1. This is most likely due to cytoplasmic contamination. The data in Table 2 were obtained from preparations that were carefully prepared and washed before assay. Perhaps more relevant to dosage studies of MOD-2 in kidney is the fact that the reaction consistently deviated from linear kinetics after about 2 min, unlike the heart extracts. Thus, determinations were made in our study on the basis of initial velocities only.

Eicher and Coleman¹² have clearly demonstrated the expected dosage effect on MOD-2 expression in male mice carrying three copies of MOD-2 due to an unbalanced translocation [T(X;7)1 Ct]. Their data on females, however, are not in agreement with simple hypotheses assuming a linear gene dosage effect due to the random inactivation of one X chromosome. The basis of these inconsistencies with the linear gene dosage–gene product relationship in the MOD-2 system remains to be explained.

This research was sponsored by the Department of Energy under contract with the Union Carbide Corporation.

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Received 9 September; accepted 19 December, 1977.

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Naturally occurring respiratory deficient *Candida slooffii* strains resemble *petite* mutants

CYTOPLASMICALLY inherited respiratory deficient mutants termed *petites*, were first described in baker's yeast over 20 years ago¹. Since then in laboratory studies several species of yeast have been shown to give rise to respiratory deficient mutants^{2,3}. However, although some respiratory deficient (obligatory fermentative) yeasts have been found in nature³, it is not clear whether these species are the result of cytoplasmic (*petite*) or chromosomal lesions⁴ because no suitable genetic tests are available. Nevertheless it is known that *petite* mutants of both *Saccharomyces cerevisiae*⁵ and *Torulopsis glabrata*⁶ have large deletions from their mitochondrial DNA with each independent isolate having a unique circular DNA size profile which can be regarded as a 'fingerprint'. By contrast chromosomal or segregational mutants have intact and functional mitochondrial DNA, as shown by genetic tests^{3,7,8}. Therefore it seemed possible that the type of lesion in naturally occurring respiratory deficient species could be determined by circular DNA profile analysis. Using this method, we show here that three independently isolated strains of *Candida slooffii* resemble *petite* mutants.

C. slooffii is a naturally occurring respiratory deficient yeast originally obtained from the anaerobic environment of horse intestine but which has since been found in the guts of several other animals⁹. This yeast has been confirmed as lacking respiration; in addition, it has been shown to lack cytochrome *aa₃*, to have poorly defined mitochondrial profiles¹⁰ and to never revert to respiratory competent forms.

Independently isolated *C. slooffii* strains 2419, 2783 and 4068 were obtained from Centraalbureau voor Schimmelcultures, Delft. Total cellular DNA was prepared as detailed in Fig. 1 and analysed by buoyant density centrifugation. Main band DNA of strain 2419, the type strain and representative of all three strains, has a buoyant density of 1.6915 g cm⁻³ in agreement with a previous report for *C. slooffii*¹¹. On the heavy side of the main band there is a small peak of density 1.7065 g cm⁻³ which is also present in the other two strains. By analogy with *S. cerevisiae* this peak probably represents ribosomal DNA¹². On the lighter side of the main band there is a peak of density 1.6785 g cm⁻³. This light buoyant density DNA is the only component present in particles which band in a sucrose gradient in an identical position to similarly prepared mitochondrial profiles from *petite* mutants of *S. cerevisiae*¹³.

Additional evidence supporting the belief that the light buoyant density DNA in *C. slooffii* is mitochondrial in origin comes from studies using ethidium bromide. On treatment of strain 2419 with this drug, cultures are obtained which completely lack the light buoyant density component (Fig. 1). Similarly the intercalative dye, ethidium bromide, causes the complete elimination of mitochondrial DNA in *S. cerevisiae*^{14,15} and *T. glabrata*⁶. Indeed, drug-induced selective elimination of light buoyant density DNA in *petite* positive yeasts is a widely accepted diagnostic criterion for mitochondrial DNA.

When circular DNA is prepared from mitochondrial-like particles of the three *C. slooffii* cultures as detailed in Fig. 2 a single light buoyant density peak is found in each of the strains. However, each circular DNA has a different buoyant density which ranges from 1.680 to 1.674 g cm⁻³.

Confirmation that these circular DNAs are different was obtained by electron microscopy which showed that each

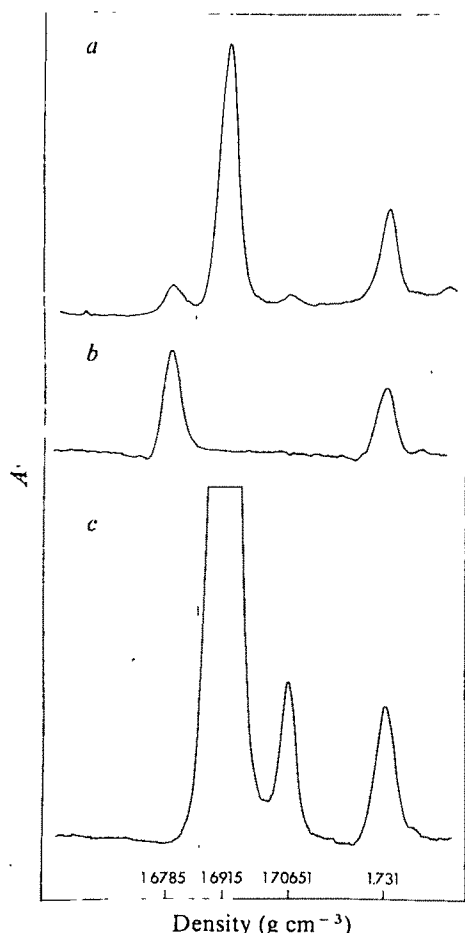


Fig. 1 Buoyant density traces in CsCl of DNA from *C. slooffii* strain 2419. *a*, Total cellular DNA; *b*, DNA from purified mitochondria-like particles and *c*, total cellular DNA from a culture isolated after euflavine treatment. The third gradient, containing 8.7 μ g DNA, was deliberately overloaded in an attempt to detect the light buoyant density component. Cultures were grown at 37 °C in 2% glucose, 1% yeast extract (Oxoid), 0.5% peptone (Oxoid), mineral salts¹⁹ and vitamins²⁰. Cells were converted to spheroplasts by incubation in support buffer (0.9M sorbitol, 0.02M imidazole-HCl pH 6.4, 1 mM EDTA) using a cell wall digesting enzyme mixture (1 g Driselase (Kyowa Hakko Kogyo) to 50 g wet weight of cells). Driselase was removed by two washes in support buffer and cells were lysed by the addition of sarkosyl to a final concentration of 2%. Lysed preparations were added to CsCl-ethidium bromide (as detailed previously¹³) and centrifuged for 44 h, at 10 °C, 48,000 r.p.m. in a Beckman 50 Ti rotor. DNA was visualised by ultraviolet excited fluorescence of intercalated ethidium bromide, extracted by side puncture of the tube and the dye removed by extraction with isoamyl alcohol. Buoyant densities were determined in the Spinco Model E analytical ultracentrifuge operated at 44,000 r.p.m. using *Micrococcus luteus* DNA of density 1.731 g cm⁻³ as a marker. Mitochondrion-like particles were obtained by gentle breakage of spheroplasts by passage through a French press at 2,000 lb/in². After removal of unbroken cells, debris and nuclei by successive centrifugations at 1,200g and 2,500g for 5 min in the Sorvall SS 34 rotor mitochondrion-like particles were collected by centrifugation at 8,500g for 20 min. The sedimented particles were resuspended in support buffer and layered onto linear sucrose gradients (25 g sucrose + 75 ml of 0.01 M EDTA, 0.01 M Tris-HCl pH 7.0; 55 g sucrose + 45 ml solvent) which were centrifuged at 25,000 r.p.m. for 1 h at 10 °C in the Beckman SW27 rotor. The particulate band in the middle of the gradient was extracted by side puncture, diluted with 3 volumes of support buffer and the particles collected by centrifugation. DNA was then isolated as outlined above. Treatment of *C. slooffii* strain 2419 with euflavine was performed as previously detailed^{1,4} by drawing a loop of culture across a drop of the drug (10 mg ml⁻¹) which had dried into agar containing the medium described above. After 2 d at 37 °C cells from the margin of growth were streaked away from the drug. This procedure ensures that individual colonies are obtained which arise from cells exposed to the highest concentration of euflavine compatible with survival.

preparation has a unique circular DNA size profile (Fig. 3). Strain 2419 has a clearly defined major length class at 0.56 μ m with smaller peaks at 1.28 and 1.76 μ m possibly representing oligomeric forms. A similar situation occurs in strain 4068 with a size class at 0.70 μ m and a possible dimeric form at 1.36 μ m. However, strain 2783 has an entirely different profile which is probably heterogeneous for several overlapping size classes. These results showing that each of the three isolates of *C. slooffii* has circular DNA differing both in buoyant density and circular size profile are therefore directly analogous to results obtained from independently derived *petite* mutants of both *S. cerevisiae* and *T. glabrata* as discussed above.

Although these results cannot exclude the possibility that the three strains of *C. slooffii* are double mutants harbouring chromosomal lesions for respiratory deficiency they do indicate that the mitochondrial DNA has suffered large deletions and that each strain is independently derived. In these respects they resemble true *petite* mutants.

On the basis of the above results, we suggest that *petite* mutants of many yeast species, rather than being laboratory curiosities, may indeed be widespread in habitats which do not select for respiratory competence. Other naturally occurring respiratory deficient yeasts which are likely *petite* mutants are *Torulopsis pintoalopesii*¹¹, *Torulopsis lactis-condensi*^{2,16} and *Schizosaccharomyces japonicus* variety *versatilis*^{2,17,18}.

Our results also indicate that the *C. slooffii* strains continue to harbour DNA that has no apparent function and which can be eliminated without affecting their viability. Although the continued replication of functionless DNA seems anomalous the cells probably have no means of preventing it.

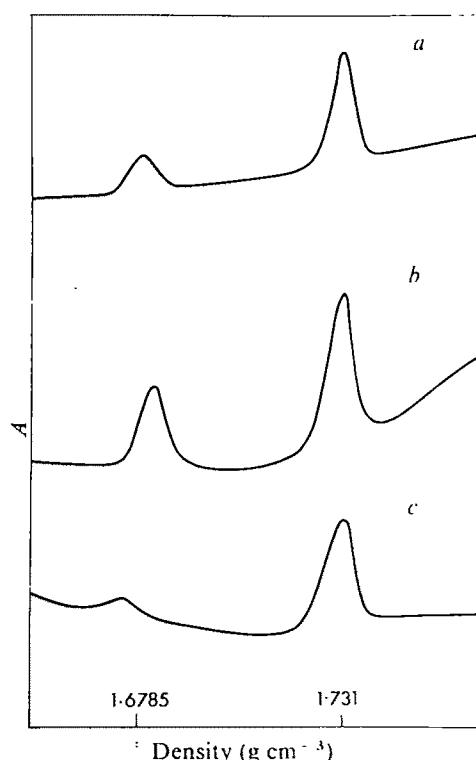


Fig. 2 Buoyant density traces in CsCl of closed circular DNA from three strains of *C. slooffii*. Circular DNA was obtained as detailed previously^{13,21} by centrifugation in CsCl-ethidium bromide of a sarkosyl lysate of a membrane enriched fraction obtained after breaking cells in a Braun homogeniser. The closed circular DNA was freed from ethidium bromide by extraction with isoamyl alcohol, dialysed against 0.015 M NaCl, 0.0015 M Na citrate pH 7.0 and buoyant densities determined in the Spinco model E analytical ultracentrifuge. Circular DNAs from strains 2419 (*a*), 2783 (*b*) and 4068 (*c*) have buoyant densities of 1.6785, 1.680 and 1.674 g cm⁻³ respectively. *Micrococcus luteus* DNA of density 1.731 g cm⁻³ is a marker.

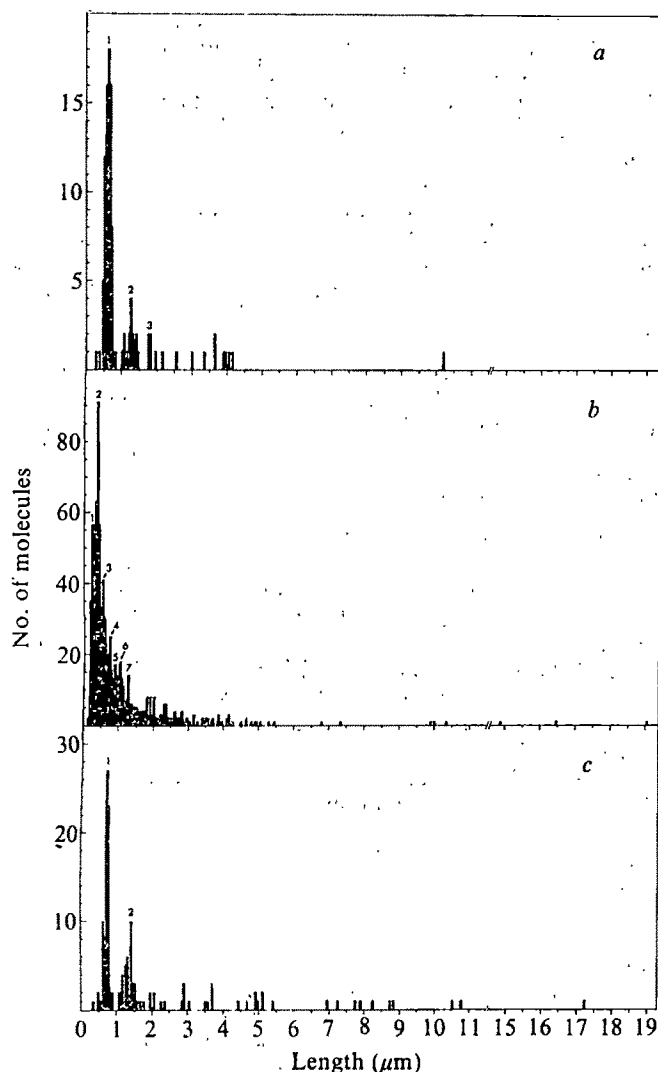


Fig. 3 Length distributions of relaxed circular DNA molecules from the closed circular DNA preparations of the three *C. slooffii* strains as observed in the electron microscope. The peaks indicated by the numerals have numbers and mean lengths of: a, strain 2419; 1, $n = 77$, $l_{\text{mean}} = 0.56 \pm 0.08 \mu\text{m}$; 2, $n = 13$, $l_{\text{mean}} = 1.28 \pm 0.09 \mu\text{m}$; 3, $n = 4$, $l_{\text{mean}} = 1.76 \pm 0.03 \mu\text{m}$; b, strain 2783; 1, $n = 119$, $l_{\text{mean}} = 0.19 \pm 0.04 \mu\text{m}$; 2, $n = 211$, $l_{\text{mean}} = 0.34 \pm 0.04 \mu\text{m}$; 3, $n = 97$, $l_{\text{mean}} = 0.50 \pm 0.03 \mu\text{m}$; 4, $n = 75$, $l_{\text{mean}} = 0.67 \pm 0.05 \mu\text{m}$; 5, $n = 44$, $l_{\text{mean}} = 0.84 \pm 0.04 \mu\text{m}$; 6, $n = 49$, $l_{\text{mean}} = 1.02 \pm 0.05 \mu\text{m}$; 7, $n = 26$, $l_{\text{mean}} = 1.20 \pm 0.04 \mu\text{m}$; c, strain 4068; 1, $n = 72$, $l_{\text{mean}} = 0.70 \pm 0.06 \mu\text{m}$; 2, $n = 29$, $l_{\text{mean}} = 1.36 \pm 0.08 \mu\text{m}$. The procedure for visualisation of the DNA was as described previously²¹.

We thank K. Williams and W. Hayes for critical comments. K.W. acknowledges support from the Australian Research Grants Committee and H.A. the award of an Australian Government Postgraduate Scholarship.

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Received 10 November 1977; accepted 3 January 1978.

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Toxicity of diphtheria toxin for lymphoblastoid cells is increased by conjugation to antilymphocytic globulin

THERE is a pressing need in medicine for cytotoxic agents with tissue specificity. It may be possible to produce such agents by conjugating cytotoxic materials, themselves lacking inherent specificity, to antibodies directed against surface antigens peculiar to target cells. Various agents, including cytotoxic drugs¹⁻⁷, radioactive materials⁸, radiosensitising agents⁹ and enzymes¹⁰⁻¹¹ have been covalently coupled to antitumour antibodies in attempts to produce such antitumour agents but so far these have not consistently produced cures in tumour-bearing animals. We, like others¹²⁻¹⁶, have chosen to use diphtheria toxin because we consider that the paucity of tumour-specific antigens, accentuated, perhaps, by their restricted accessibility in solid tumours, necessitates the use of the most potent cytotoxins available.

The mode of action of diphtheria toxin is thought to involve the entry into a cell of the toxic 'A' chain following an interaction of its 'B' chain with receptors on the plasma membrane^{17,18}. Once inside the cell the 'A' chain terminates protein synthesis by enzymatic inactivation of peptidyl-tRNA translocation factor, EF-2 (ref. 19).

Moolten *et al.*¹⁴ covalently coupled diphtheria toxin to antibodies directed against SV40-induced antigens and produced a conjugate that exhibited a 2.2-fold greater 'specific toxicity' for SV40-transformed tumour cells *in vitro* than a conjugate of normal immunoglobulin. In spite of this small differential toxicity, the conjugate prolonged the life of, and occasionally cured, hamsters bearing an SV40-induced lymphoma. The fact that these workers used glutaraldehyde to couple toxin to antibody might explain why the conjugate only possessed marginal differential toxicity *in vitro* since this coupling agent is likely to have formed intra-chain bonds within the toxin molecule, thus preventing the 'A' chain from dissociating from the 'B' chain and penetrating into the cell. Here we report a new chemical coupling procedure which avoids the formation of intra-chain bonds in the toxin molecule. Using this method we have prepared a conjugate of diphtheria toxin and antilymphocytic globulin which can kill lymphoid cells at less than one-thousandth of the dose necessary for nonconjugated toxin.

Anti-human lymphocytic globulin (ALG) was raised by injection of a human lymphoblastoid cell line WRL7 into a horse and the IgG fraction of the serum was purified by conventional methods²⁰. The target cell was CLA4, a human lymphoblastoid cell line initiated by Dr M. Steel from cord blood leukocytes. The limit of binding of the ALG to the CLA4 cells as detected by indirect immunofluorescence was 0.012 mg ml⁻¹.

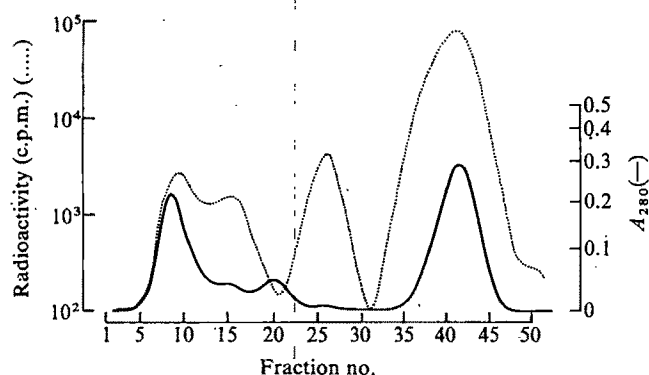


Fig. 1 Gel filtration on Sephadex G-150 of the products of reaction between diphtheria toxin and the chlorambucil-ALG conjugate. The eluate was monitored by absorption at 280 nm (—) and by radioactivity (c.p.m.) (....) derived from the ¹²⁵I-radiolabelled diphtheria toxin. Between fractions 20 and 31 was recovered a peak of radioactivity without concomitant absorbance due to the tendency of ¹²⁵I-radiolabelled toxin molecules to dimerise. Chlorambucil (7.5 mg, Wellcome) was dissolved in 0.3 ml of a 1.15% (v/v) solution of triethylamine in tetrahydrofuran and to this was added 0.3 ml of a 1.13% (w/v) solution of butyl chloroformate in dioxan. After stirring at 4 °C for 30 min a precooled solution of ALG (50 mg) in 5 ml of borate buffer (0.05M, pH 9) containing 1.7% NaCl and 1.4 ml dioxan was added. After stirring at 4 °C for a further 90 min, the solution was applied to a Sephadex G-25 column (1.6 × 40 cm) maintained at 4 °C and eluted with saline-borate buffer. The protein eluate was concentrated to 2.5 ml by ultrafiltration (Amicon) at 4 °C and 1 ml of the concentrate added to 1.0 ml of diphtheria toxin (25 mg ml⁻¹) and 0.05 ml of ¹²⁵I-radiolabelled toxin. The mixture was stirred at 25–30 °C for about 30 h, centrifuged, and 0.7 ml of the supernatant applied to the Sephadex G-150 (superfine) column (1.6 × 90 cm).

Diphtheria toxin was purified from a filtrate of a culture of *Corynebacterium diphtheriae* by ammonium sulphate fractionation and chromatography on DEAE-Sephadex and Sephadex G-100²². The leucine incorporation by human fibroblasts was reduced by 50% following treatment with 10⁻⁸ mg ml⁻¹ toxin for 24 h. Comparable inhibition in human peripheral lymphoid cells was obtained using 10⁻⁴ mg ml⁻¹ toxin.¹²⁵I-labelled toxin was prepared²¹ and mixed with unlabelled toxin to provide a ready means of detection and assay.

To prepare the conjugates, chlorambucil was converted to a mixed anhydride which was reacted with ALG at 4 °C (detailed in legend to Fig. 1). The resulting ALG-chlorambucil conjugate was freed from unreacted chlorambucil by filtration on Sephadex G-25 and was then mixed with diphtheria toxin. The temperature was raised to 25–30 °C to activate the mustard groups of the chlorambucil, which could then react with toxin to produce ALG-toxin conjugates or with ALG to produce polymers. This procedure avoids the formation of the toxin polymers that are formed when conventional coupling methods are used.

The products of the coupling reaction were subjected to gel filtration on Sephadex G-150 with the results illustrated in Fig. 1. The constitution of the fractions is summarised in Table 1. The bulked fractions 13–16 were as capable as native ALG of binding to CLA4 cells (immunofluorescence titre 0.012 mg ml⁻¹ ALG) and were used in the subsequent testing. By comparison, fractions 4–12 possessed inferior ability to bind to, and a lesser cytotoxic effect on, CLA4 cells.

CLA4 cells were treated with diphtheria toxin or ALG-toxin conjugate for 1 h, washed five times to remove non-bound material and incubated for a further 23 h. Continuous incubation for 24 h in the presence of the agents was also carried out. Toxic effects were judged by the reduction in the incorporation of ³H-leucine into cellular protein during a 16 h pulse with the labelled amino acid at the end of the incubation period. The results of the experiment are shown in Fig. 2.

Given a 24 h exposure to 10⁻⁶ mg ml⁻¹ toxin the leucine incorporation into CLA4 cells was reduced by only 50% and on 1 h exposure the reduction was around 25% at the highest concentration of toxin used (10⁻³ mg ml⁻¹). By contrast, the ALG-toxin conjugate reduced leucine uptake by 50% at toxin concentrations of 10⁻⁶ mg ml⁻¹ on both 1 h and 24 h exposure. Thus by attachment to ALG, the cytotoxic effect of diphtheria toxin on 1 h exposure to CLA4 cells was increased more than 1,000-fold. ALG alone, although causing agglutination, did not affect leucine uptake. When ALG and toxin were used simultaneously but not chemically coupled no increase in toxicity occurred, thus the effects we describe are not due to synergism between toxin and antibody as has been demonstrated for other drug-antibody combinations^{23–27}.

In order to confirm that the increased toxicity of ALG-toxin compared to toxin alone was mediated by an antigen-antibody reaction an experiment was performed in which normal horse IgG, with no detectable ability to bind to CLA4 cells, was used in place of the ALG. The results are shown in Fig. 3. The IgG-toxin conjugate (molecular weight 180,000–240,000) was in fact less toxic for CLA4 cells than free diphtheria toxin, the effect being most apparent after exposure for 24 h when the difference in toxicity was around 50-fold.

There are several mechanisms which could account for the increase in toxicity of diphtheria toxin for CLA4 cells after its conjugation to ALG. One is that CLA4 cells possess far more antigenic sites for combination with antibody than receptors for toxin, so that the conjugate effectively concentrates toxin on the cell membrane. A numerical difference of this kind may not entirely account for the effect as cells washed after exposure to toxin for 1 h subsequently develop less severe signs of cytotoxic action than cells exposed for 24 h, whereas the toxic effects of the conjugate are not reduced by washing; this observation is compatible with the notion that the affinity of toxin for its receptor is less than that of antibody for antigen. Lastly, the fate of toxin taken into cells by pinocytosis is thought to be proteolysis by lysosomal enzymes^{27–28}; however, if a mechanism exists whereby the

Table 1 Analysis of the proteins eluted from Sephadex G-150 in Fig. 1

Fractions	Approximate molecular weight range	ALG* (mg)	Toxin (mg)	Chlorambucil* (mg)	Chlorambucil:ALG (molar ratio)	ALG:DT (molar ratio)	Predominant molecular species
4–12	> 240,000	3.02	0.23	0.066	10.8	5.5	High molecular weight conjugates and ALG polymers
13–16	180,000–240,000	0.58	0.09	0.008	7.1	2.8	ALG-toxin (1:1), ALG and ALG-ALG
17–21	140,000–180,000	0.75	0.038†	0.010	6.7	8.5	ALG-toxin (1:1), ALG
35–46	50,000–80,000	—	8.09	—	—	—	Toxin

*Concentrations of ALG and chlorambucil calculated from spectrophotometric data at 278 and 261 nm (refs 29, 30).

†This figure is inflated by the presence of ¹²⁵I-toxin dimers (see legend to Fig. 1).

undegraded toxin can escape from an endocytic vesicle into the cytosol then the combination of divalent antibody to the toxin could promote its internalisation by capping²⁸.

The decrease in toxicity found with the IgG-toxin conjugate for CLA4 cells is presumably due to steric hindrance of the IgG with the toxin-receptor interaction. This is supported by our finding that both ALG-toxin and IgG-toxin were 50-fold less effective than toxin alone at inhibiting leucine uptake by human fibroblasts *in vitro*, a cell type to which neither ALG nor IgG bound. The reduction in nonspecific toxicity of diphtheria toxin after conjugation to immunoglobulin might possibly be reflected by a reduction in undesirable side effects *in vivo*.

By using a chemical coupling procedure designed to eliminate the risk of producing intra-chain linkages in the toxin molecule thereby placing no restriction on the freedom of the 'A' chain to penetrate the cell, we have produced conjugates with greatly enhanced toxicity for the target. We believe that this represents

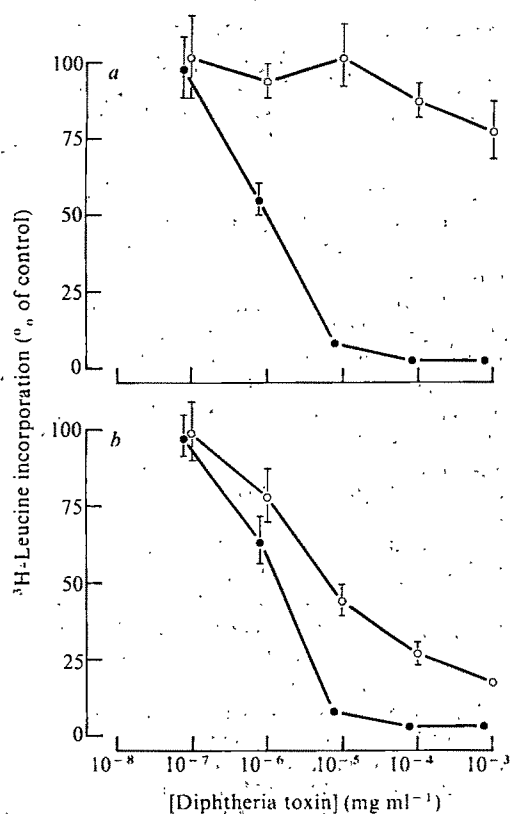


Fig. 2 The effect of diphtheria toxin alone (○) or conjugated to ALG (fractions 13 to 16) (●) on the ³H-leucine incorporation by CLA4 cells, expressed as % of incorporation by untreated cultures. The CLA4 cells were suspended in Hanks' balanced salt solution containing 10% heat inactivated foetal calf serum; this medium is free from glutamine, a substance known to interfere with the cytotoxic activity of diphtheria toxin³¹. The suspension (1 × 10⁶ cells ml⁻¹) was distributed in 200 μl volumes into the wells of 96-well flat-bottomed microplates (Linbro). The cells were either *a*, incubated with toxins for 24 h at 37 °C or *b*, incubated with toxins for 1 h before washing five times and resuspending into fresh Hank's-serum medium for a further 23 h incubation. All cultures were resuspended after 24 h into 50:50 Dulbecco's-RPMI medium supplemented with 10% serum, glutamine (3 mM) and antibiotics and were then pulsed for 16 h with 1 μCi per culture of ³H-leucine (Amersham Radiochemicals, TRK 170, 58, Ci mmol⁻¹). Leucine uptake was measured as previously described³². Toxin added simultaneously with ALG (2.8 molecules ALG per molecule of toxin, equivalent to the ALG:toxin ratio in conjugate 13-16) had identical toxicity to that described for toxin alone in the figure. Vertical lines in the figure represent one standard deviation on geometric mean of triplicate determinations, unless smaller than the points as plotted.

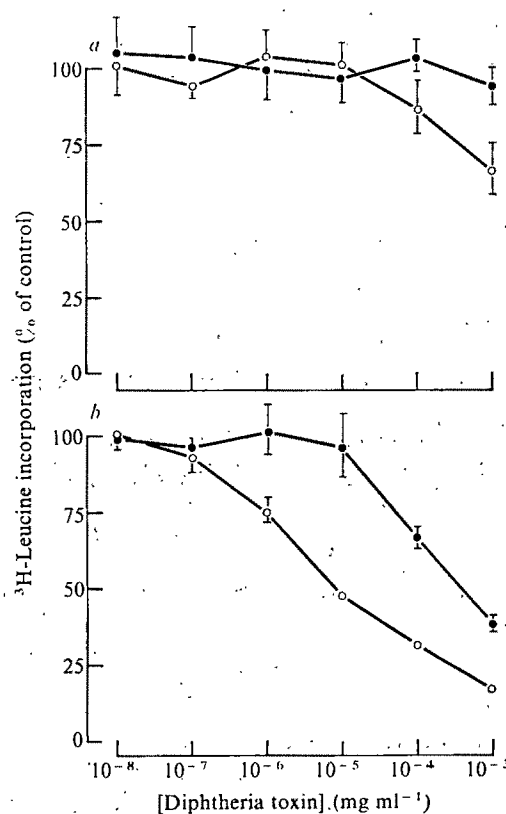


Fig. 3 The effect of diphtheria toxin alone (○) or conjugated to normal horse IgG (molecular weight 180,000-240,000) (●) on the ³H-leucine incorporation by CLA4 cells, expressed as % of incorporation by untreated cultures. The CLA4 cells were exposed to the toxins for *a*, 1 h or *b*, 24 h. Other details as for Fig. 2.

a useful step towards the design of new chemotherapeutic agents.

We thank Dr B. Mikulski for her help in maintaining the CLA4 line and Mr J. S. Courtenay for supplying the antiserum. Work at the Chester Beatty Research Institute (Institute of Cancer Research: Royal Cancer Hospital) is supported by grants from the MRC and the Cancer Research Campaign. D.C.E. is an external member of the staff of the Wellcome Foundation. C.A.H. is supported by NCI grant no. ICM/43736.

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Role of cyclic GMP in the action of heat-stable enterotoxin of *Escherichia coli*

ENTEROTOXIGENIC strains of *Escherichia coli* elaborate two enterotoxins, a heat-labile toxin (LT) and a heat-stable toxin (ST), which cause diarrhoeal disease in humans¹; ST-producing *E. coli* cause diarrhoea in adult volunteers² and have been associated with epidemic diarrhoea in a nursery for the newborn³ and with sporadic adult diarrhoea among North American tourists to Latin America⁴ and the Navajo people in Arizona⁵. LT-producing strains are identified by the ability of culture filtrates to cause fluid accumulation in rabbit ileal loops at 18 h (ref. 6) or by morphological alteration of Chinese hamster ovary (CHO) cells⁷ or of Y-1 adrenal cells⁸. ST-producing strains are identified by the ability of culture filtrates to cause earlier fluid accumulation in rabbit ileal loops (peak accumulation at 6 h (ref. 6), or by fluid accumulation in the gut of the suckling mouse at 3 h (refs 9, 10). LT acts in a manner similar to cholera toxin (CT) by activating adenylate cyclase¹¹. The mechanism of action of ST is unknown, however. Culture filtrates of a strain of *E. coli* that produced both LT and ST caused immediate net fluid secretion in canine jejunal segments without the 1-h delay characteristic of the response to CT^{12,13}. In addition, culture filtrates caused an immediate increase in canine jejunal adenylate cyclase activity as measured by enzymatic generation of P³²-cyclic AMP from P³²-labelled ATP¹³, also in contrast to the delay in appearance of increased adenylate cyclase activity following exposure to CT¹². The possibility that the early effect of ST was mediated by changes in cyclic nucleotide concentrations was investigated; culture filtrates of ST-producing strains of *E. coli* caused increased cyclic GMP concentrations in rabbit intestinal tissue and

Fig. 1 Dose-response effect of 8BrGMP on suckling mouse fluid secretion at 60 min. Points represent means \pm s.e.m. of three or more groups of two to five mice representing a total of seven or more mice. Mice, aged 2-4 d were inoculated intragastrically with 0.1 ml of solutions containing 0.12-5 μ mol 8BrGMP per mouse.

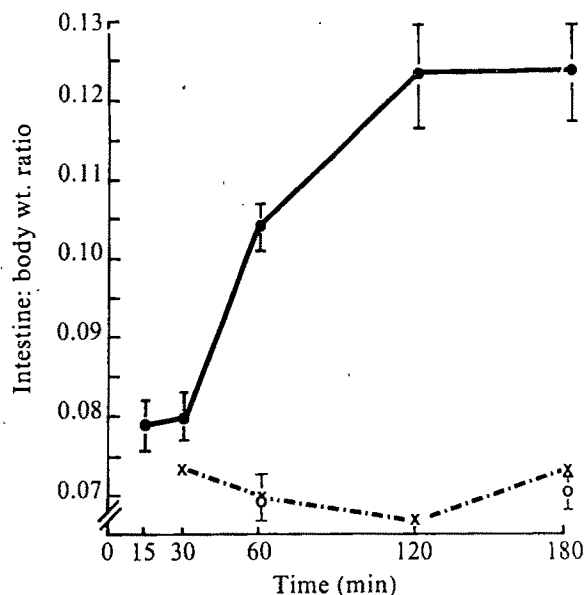
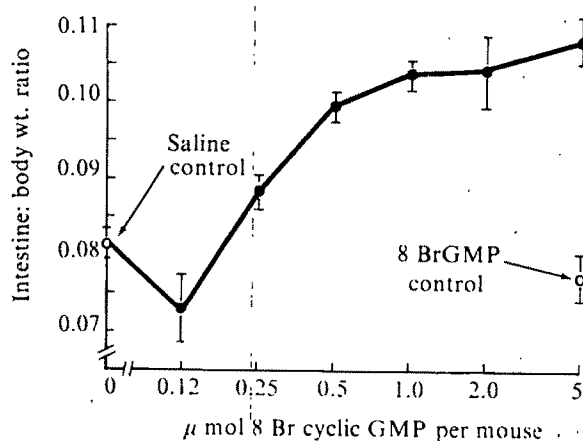


Fig. 2 Time course of 8BrGMP effect on suckling mouse fluid secretion. Points represent means \pm s.e.m. of three or more groups of two to five mice representing a total of seven or more mice. ●, 8BrGMP; 1 μ mol per mouse; ×, 0.1 μ mol 8BrGMP per mouse; ○, control 8BrGMP (1 or 10 μ mol per mouse).

the cyclic GMP analogue 8BrGMP mimicked ST in magnitude and time course of intestinal fluid accumulation in both rabbits and suckling mice.

Two *E. coli* strains (B30-5 and B44¹⁴) which produced only ST were used and rabbit intestinal tissue levels of cyclic AMP and cyclic GMP were determined in biopsies taken after inoculation of ligated intestinal loops with culture filtrates of these strains. Mean mucosal cyclic GMP concentrations in intestinal segments exposed to filtrates of strains B30-5 and B44 for 20 min were over threefold greater than in control loops ($P < 0.02$, Table 1); cyclic AMP levels were not significantly different.

To determine if cyclic GMP analogues could produce intestinal fluid accumulation in the rabbit, ligated intestinal segments were inoculated with 8BrGMP; controls loops were inoculated with 8-Br-5'-GMP (8BrGMP). Ratios of intestinal fluid to segment length were significantly greater in loops exposed to 8BrGMP than in controls (Table 2). This volume/length ratio approximates the reported effect of ST at 2 h in this model⁶. As fluid secretion might have occurred as a result of increased tissue cyclic AMP levels secondary to inhibition of cyclic AMP phosphodiesterase by 8BrGMP¹⁵, the effect of 8BrGMP, 8BrGMP, and water on intestinal cyclic AMP levels was investigated. At 20 min and 2 h after loop inoculation, tissue samples were homogenised in trichloroacetic acid, extracted, and purified¹⁶ for determination of cyclic AMP levels. Tissue cyclic AMP levels in loops exposed to 1.25 mM 8BrGMP were not significantly different from control loops exposed to either 1.25 mM 8BrGMP or water at 20 min (24 ± 1 compared with 28 ± 2 pmol per mg protein) or at 2 h (24 ± 1 compared with 27 ± 2 pmol per mg protein). As in studies shown in Table 2, intestinal loop fluid accumulation expressed as volume/length ratios was significantly greater in loops exposed to 8BrGMP ($P < 0.02$) in these experiments.

To determine if cyclic GMP analogues would cause fluid accumulation in the suckling mouse assay used for detecting ST-producing strains of *E. coli*⁹⁻¹⁰, the response of suckling mice to 8BrGMP and 8BrGMP was investigated. The dose-response curve at 1 h (Fig. 1) shows that doses of 0.25 μ mol or greater produced significant fluid accumulation in the mouse gut compared with either normal saline or 5 μ mol 8BrGMP controls ($P < 0.05$). Using 1 μ mol 8BrGMP per mouse, the

Table 1 Rabbit intestinal cyclic nucleotide concentrations after 20 min exposure to culture filtrates of ST-producing and control *E. coli*

	ST strains	Controls
Cyclic GMP (fmol per mg protein)	1991 ± 422*	572 ± 62*
Cyclic AMP (pmol per mg protein)	34 ± 15†	18 ± 2†

Values are means ± s.e.m. of 7 observations with ST strains and 10 observations with control strains. Filtrates were prepared from 18 h cultures grown in casamino acids-yeast extract salts broth (CA-YE)¹⁹ in a roller drum at 37 °C; volumes of 1 ml were injected into 4–5 cm ligated segments of rabbit ileum as previously described⁹. Control loops were inoculated with sterile CA-YE broth or culture filtrates from nontoxicogenic *E. coli* strain 10405. Full thickness biopsies of each loop obtained 20 min after inoculation were promptly homogenised in 2 ml of chilled 6% trichloroacetic acid (TCA). Specimens were stored at –20 °C prior to centrifugation for removal of the pellet for protein determination by the Lowry method¹⁵. After ether extraction of the supernatant fraction, cyclic AMP and cyclic GMP concentrations were determined by radioimmunoassay^{16,17}.

* $P < 0.02$, student's *t* test.

†Not significant change.

time course (Fig. 2) indicated that significant fluid accumulation occurred by 60 min ($P < 0.01$) and became maximal by 2 h.

The response of both rabbit and suckling mouse intestine to 8BrGMP which mimics the action of culture filtrates of ST-producing strains of *E. coli* in these models and the significant increase in rabbit intestinal cyclic GMP levels after exposure to culture filtrates of ST-producing strains suggest that intestinal fluid accumulation induced by ST may be mediated through increases in intestinal guanylate cyclase activity. This possibility is consistent with the previous observation that canine intestinal adenylate cyclase activity was promptly elevated following exposure to culture filtrates containing ST¹³; in these experiments, adenylate cyclase activity was measured using the Krishna assay²⁰ which measures enzyme activity as a function of conversion of radiolabelled ATP to cyclic AMP. However, activated guanylate cyclase can convert ATP to cyclic AMP²¹ and might therefore be measured as adenylate cyclase activity in the Krishna assay.

That cyclic GMP might play an important part in the mediation of intestinal fluid secretion in humans with diarrhoea caused by ST-producing strains of *E. coli* is further supported by previous observations that a 2 mM solution of cyclic GMP increases the short-circuit current in the isolated toad bladder after a 3- to 5-min lag period following application to the serosal side of the membrane²². However, other studies have demonstrated that α -adrenergic and cholinergic agents may increase rabbit intestinal mucosal cyclic GMP levels while also increasing Na⁺ and Cl[–] absorption rather than secretion, but exogenous 8BrGMP failed to reproduce these effects²³. The possibility exists that multiple pools of cyclic GMP may be present in intestinal mucosal cells, as suggested for cyclic AMP²¹, and that changes in one of these compartments may effect electrolyte secretion. Further support for a possible secretory role for cyclic GMP is provided by the observation

that after cholinergic stimulation of guinea pig pancreatic islet cells, cyclic GMP increases promptly in both intracellular and extracellular phases, possibly mediating secretion of amylase²⁵. The location of intestinal cyclic GMP and guanylate cyclase activity has been demonstrated to be in the intestinal mucosal microvilli of the rat^{26,27}.

The association of increased intestinal mucosal concentrations of cyclic GMP with ST-induced intestinal secretion and the ability of 8BrGMP to mimic the effects of ST without concomitantly increasing tissue cyclic AMP levels in standard animal models suggests a role for cyclic GMP in the pathogenesis of ST-associated diarrhoea. These findings may suggest new approaches to the pharmacological control of diarrhoea caused by ST-producing strains of *E. coli*.

Note added in proof: Since this manuscript was submitted for publication, it has been learned that M. Field, L. H. Graf, Jr, W. J. Laird and P. L. Smith have found that an ST preparation increases electrical potential difference and guanylate cyclase activity in rabbit ileal mucosa (submitted for publication).

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Table 2 Rabbit intestinal fluid accumulation after 2 h of exposure to 1.25 mM 8BrGMP and control (8BrGMP)

	Volume/length ratio (ml cm ^{–1})	
8BrGMP	0.29	0.03
8BrGMP	0.17	0.02

Values represent means ± s.e.m. of 10 observations with 8BrGMP and 6 observations with 8BrGMP. Student's *t* test: $P < 0.01$. One ml of 1.25 mM 8BrGMP was inoculated into approximately 4-cm ligated rabbit intestinal segments; controls were inoculated with 1.25–10 mM 8BrGMP. At 2 h, rabbits were killed with intravenous sodium pentobarbital and the ligated small intestinal segments removed. Segment lengths and fluid volumes were measured and volume/length ratios determined.

Induction of IgG production in human B lymphoblastoid cell lines with normal human T cells

THYMUS-DERIVED (T) lymphocytes play a critical part in the induction of B lymphocytes to antibody production¹, especially for conversion of IgM to IgG response². In humans, the presence of T lymphocytes is also essential for the induction of IgM or IgG production by pokeweed mitogen (PWM)-stimulated B lymphocytes^{3,4}. In many experiments it has been

shown that antigen-specific⁵ or nonspecific⁶⁻⁸ soluble factors from T cells, together with antigen or other inducers, for example, anti-immunoglobulin antibody, acting on B-cell surface receptors⁹, can also provide the stimulus for Ig production in B cells. However, the chemical nature of a B-cell acceptor for the T-effector molecule, the biochemical events responsible for the differentiation of B cells to Ig-producing cells, and the mechanism of the switch of transcription from μ chain to γ chain genes under the influence of T cells remain largely unknown. Heterogeneity of B-cell population in spleen, lymph node and blood has hindered the molecular analysis of immune phenomena. In this situation, B lymphoblastoid cell lines may be useful models for such analysis, since they may be arrested at certain phases of their differentiative history and if influenced by T cells or T-cell factors might permit incisive analysis of induction and switching of gene action at

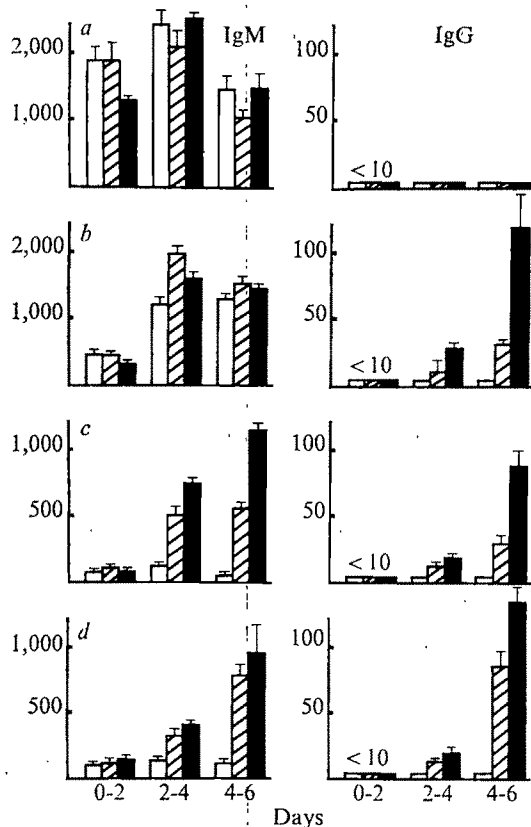


Fig. 1 Induction of IgG production in human B-cell lines by normal T cells and macrophage factors. B-cell lines were as described previously^{16,17}. They were maintained in RPMI 1640 medium containing 10% foetal calf serum and 5×10^{-5} M 2-mercaptoethanol. Experimental cultures were initiated in the same medium in triplicate in 0.2 ml volumes (Falcon 3040 microtest plates) containing 10^4 B cell lines (open bars), and where indicated 10^5 normal T cells (donor Y.N.) (hatched bars), or T cell plus 50% v/v macrophage factor (solid bars). Cultures were incubated at 37 °C in 5% CO₂. After 2, 4 and 6 d incubation, the plates were centrifuged, supernatants collected and assayed for IgM and IgG by a radioimmunoassay¹⁵. Fresh medium was added to the cultures after the samplings. T cells were purified from normal human blood lymphocytes by neuraminidase-treated sheep RBC rosette formation¹⁵, and contained less than 1% B cells (surface Ig⁺ cells by immunofluorescence). Macrophage factor was obtained from culturing J774.1 cell line¹¹ with $1 \mu\text{g ml}^{-1}$ LPS (type B, *Escherichia coli* 055:B5, Difco) for 24 h and dialysing the supernatant against culture medium. Supernates prepared without LPS or with LPS added at the end of 24 h were not active. Results are shown as IgG (right hand column) or IgM (left) ng ml⁻¹ (\pm 1 s.d.). The background sensitivity of the assays are 10 ng ml⁻¹ IgG and 20 ng ml⁻¹ IgM. Specificity is shown by failure to detect cross reactions using 10,000 ng ml⁻¹ heterologous antigen. Recovery of lymphoblast cells at day 6 was the same for cultures of B cells alone, of B cells with T cells, or of B cells plus macrophage factors, for all experiments. a, BM; b, RPMI 1788; c, Daudi; d, SB-cell line.

the molecular level. We report here the induction of IgG production or enhancement of IgM secretion in several human B lymphoblastoid cell lines with the help of normal T cells.

Five cell lines were studied for production of IgM and IgG when cultured alone, with normal human T cells, and with human T cells plus macrophage factors. Lines BM, RPMI 1788 and Daudi had IgM on the cell surface as well as in the cytoplasm when tested by immunofluorescence, and lines BM and RPMI 1788 continuously secreted IgM in the culture supernatant, but did not secrete any IgG molecules when tested by radioimmunoassay. Line SB had IgM and IgG on the surface and inside the cells, but secreted only small amounts of IgM. As shown in Fig. 1, there were several patterns of cell-line reactivity. Line BM produced only extracellular IgM even in the presence of T cells. On the other hand, coculture of lines RPMI 1788, Daudi or SB with T cells induced IgG production. In addition, T cells also significantly increased extracellular IgM levels in Daudi and SB cultures. The stimulation of IgG and IgM production induced by T cells was not due to increased growth of the cell line, since recovery of larger lymphoblastoid cells was not affected by the presence of T cells. In experiments with cell line WIL2.A3, neither IgM nor IgG was detectable in the medium in any conditions (data not shown).

We previously showed that supernates from 24-h cultures of macrophages could replace adherent cells in the nonspecific induction of mouse T killer cells to syngeneic or allogeneic tumour targets¹⁰. Supernates of the mouse macrophage line J774.1 (ref. 11), when stimulated with bacterial lipopolysaccharide (LPS), also replaced adherent cells in this system (data not shown). Macrophages are also known to produce factors stimulatory to antibody production by B lymphocytes¹²⁻¹⁴. Therefore, the J774.1 supernatant preparation following treatment with LPS was tested for stimulation of B lines. In all cases where T cell-dependent induction of IgG was observed, inclusion of the macrophage factors further stimulated IgG production about twofold. Macrophage factors alone, however, did not induce IgG production, showing that the presence of T cells is essential for the induction of IgG in these cell lines.

The IgG production observed could not have been due to contamination of the T-cell preparation with normal B cells. The T-cell population contained less than 1% of surface Ig-positive cells. No IgG or IgM was secreted when these cells were cultured alone or with PWM for 6 d at concentrations which optimally induce Ig secretion in unfractionated lymphocytes¹⁵. To further test for possible effects of normal B cell contamination, cultures were set up with RPMI 1788 cells plus purified T cells reconstituted with normal, syngeneic B cells (Table 1). The donor T cells used in this experiment do not induce RPMI 1788 to IgG production at 10^5 cell culture, but do induce them at higher numbers of T cells. Inclusion of 10^4 B cells resulted in the production of 17 ng ml^{-1} IgG at day 6. By contrast, with 4×10^5 T cells and 1×10^4 RPMI 1788 cells, $80 \text{ ng IgG ml}^{-1}$ was observed. Such amounts of IgG would

Table 1 Test for contamination of normal B lymphocytes in cell-line induction

Cell line (RPMI 1788) $\times 10^4$	T cells $\times 10^5$	B cells $\times 10^5$	IgG (ng ml ⁻¹)
1	0	0	< 8
1	1	0	< 8
1	2	0	38.5 ± 25.9
1	4	0	80.3 ± 24.9
1	1	0.1	17.3 ± 3.8
1	0	0.1	< 8

Cultures (0.2 ml) were initiated with 10^4 RPMI 1788 cells, various numbers of normal T cells (donor T.K.), and where shown, normal B cells to represent a 10% contamination of the T cells. Normal T lymphocytes were purified as in Fig. 1, and the T-depleted population was used as B cells (60% Ig⁺). Supernate IgG was determined after culture for 6 d.

Table 2 Increase of IgG-containing cells in cell lines cocultured with T cells

	% Of fluorescent-positive lymphoblastoid cells			
	Expt. 1 without T	Expt. 1 with T	Expt. 2 without T	Expt. 2 with T
SB	21	90	—	—
BM	4	22	—	—
RPMI 1788	—	—	8	22
BM	—	—	5	30

Cell lines (10⁴ per 0.2 ml) were cultured with or without 2 × 10⁵ normal T cells for 5 d. Intracellular IgG was stained with FITC-labelled F(ab')₂ fragment of rabbit anti-human γ-chain antibody. In each experiment 200–400 larger lymphoblastoid cells were counted.

require the presence of 4 to 5 × 10⁴ normal B cells, or a 10% contamination of the T-cell preparation, such contamination would have been easily detected.

Furthermore, the fluorescent staining of intracellular IgG showed an increase of IgG-containing B lymphoblastoid cells was caused by coculturing with normal T cells (Table 2). In these experiments, however, the increases of IgG-containing cells and the amounts of extracellular IgG were not parallel, that is, (1) almost 90% of SB cells were IgG-positive when cocultured with T cells but the amount of IgG produced in culture was far less than that observed in the culture of normal peripheral blood lymphocytes (PBL) with pokeweed mitogen (PWM), (2) IgG-containing cells were observed even in BM cells cocultured with T cells, in which extracellular IgG was not detected. The result suggested that IgG production was induced in these cell lines, but that these cells produced a small amount of IgG compared to normal IgG-producing cells.

As IgG production was induced by normal T cells without the presence of known T activators, it is possible that Ia-like molecules on the allogeneic B cell lines stimulated T cells to exert a helper activity. Induction of IgG in lines RPMI 1788, Daudi and SB occurred reproducibly in four experiments, as is shown in a typical experiment in Fig. 1, using two different T cell donors. However, T cells from certain donors were consistently superior to those of other donors in this assay, as shown in Table 3. All T-cell preparations showed similar helper function in pokeweed mitogen induction of self and allogeneic normal B cells. The differences in the influence of donor T-cell activity on B-cell lines may be due to the degree of matching of cell surface antigens at the major histocompatibility complex, or to the degree of stimulation by cell line Epstein-Barr virus antigens on cells of the B-cell line. Actually, in mixed lymphocytes reactions, mytomycin C-treated RPMI 1788 cells did not stimulate T cells from donors, H.K. or A.M., while T cells from donors Y.N., T.H. and T.K. showed a significant increase of ³H-thymidine uptake (stimulation

Table 3 Cell line induction by different donor T cells

Cell line (RPMI 1788) 1 × 10 ⁴	Donor T cells 2 × 10 ⁵	Macrophage factor	IgG (ng ml ⁻¹)
+	—	—	<8
+	H.K.	—	<8
+	H.K.	+	11.3 ± 6
+	Y.N.	—	21.5 ± 0.5
+	Y.N.	+	81.0 ± 40
+	A.M.	—	9.6 ± 3.2
+	A.M.	+	13.7 ± 3.2
+	T.H.	—	20.8 ± 3.1
+	T.H.	+	46.7 ± 9.8
+	T.K.	—	40.3 ± 5.6
+	T.K.	+	120.1 ± 30.9

T cells were purified from five different donors and assayed for induction of IgG production in line RPMI 1788 with or without macrophage factor, as in Fig. 1. The T cells cultured alone contained undetectable IgG (<8 ng ml⁻¹).

index 3.2–6.0) when stimulated with mytomycin C-treated 1788 for 5 d. The results may support the idea that T cells activated against Ia-like molecules on the allogeneic B-cell lines exert helper function so as to induce the switching from IgM to IgG production in B lymphoblastoid cell lines.

This experimental system should prove for the study of gene expression responsible for IgG synthesis under the influence of T cells.

This work was supported by the Japanese Ministry of Education, Science and Culture, the US-Japan Cooperative Science Program supported by NSF and JSPS, the USNIH and NCI.

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Transplantation tolerance
induced in foetal mouse thymus *in vitro*

BURNET'S suggestion that self tolerance was gained during foetal life by elimination of self reactive clones of lymphocytes¹ has gained support from studies with lymphoid chimaeras prepared by injection of either foetal or neonatal animals with histoincompatible spleen cells², by injection of irradiated adult F₁ hybrid mice with parental-type bone marrow cells³, or by fusion of eight-cell-stage embryos⁴. However, in some situations active suppression by blocking factors⁵ or by suppressor T cells⁶ may account for the lack of reactivity in apparently tolerant animals, although it is likely that these mechanisms operate only when tolerance is incompletely induced⁷. We describe here an *in vitro* model for the study of transplantation tolerance and offer evidence that active suppression by regulatory T cells is not involved. Unresponsiveness to mixed lymphocyte culture (MLC)-stimulating determinants on histoincompatible cells was induced in foetal murine thymocytes in organ culture by incubation of foetal thymus, of 14-d gestation, in contact with mitomycin-treated fragments of adult allogeneic spleen (Fig. 1).

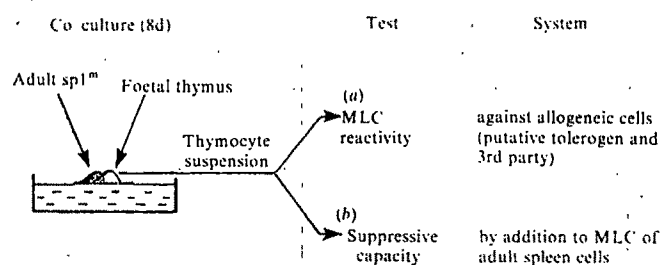


Fig. 1 Experimental protocol for tolerance induction and assay. *a*, MLC in a microculture system using 0.2 ml RPMI + 10% foetal bovine serum containing 2×10^5 each of cultured thymocytes (responder) and mitomycin-treated adult spleen cells (stimulator) in triplicate. Assayed after 4 d by an 18-h pulse of $1 \mu\text{Ci } ^{125}\text{IU}$ per culture, the c.p.m. being adjusted for decay of isotope. *b*, Suppressive effect of co-cultured thymus cells, on MLC of syngeneic adult spleen cells against the tolerated strain, tested by adding mitomycin-treated cultured thymus cells to responder cells in the ratio 1:2. The degree of suppression was expressed as percentage of MLC in the presence of the same number of syngeneic mitomycin-treated spleen cells. Suppression was controlled by sensitising suppressor cells by footpad injection of 2×10^7 allogeneic spleen cells, suppressive activity being optimal in the spleen of recipients 4 d later. Ratios of 1:1, 1:2 and 1:4 regulator: responder in MLC were plotted using the formula

$$\frac{\text{MLC ratio with activated suppressor cells} - 1}{\text{MLC ratio with control cells} - 1} \times 100 \%$$

control MLC response, when maximum suppression (to 35% of controls) was achieved using 1:2 regulator to responder ratio.

We have reported that MLC reactive cells can be generated from stem cells in organ culture*; cells reactive in allogeneic MLC against BALB/c and CBA/H adult spleen cells first appear after 4 d of culture of 14-d C57BL/6 foetal thymus. We report now that the appearance of these cells was unaffected by co-culture of foetal thymus with syngeneic spleen fragments (Fig. 1). But when foetal thymus

was co-cultured with allogeneic spleen fragments, MLC reactivity against the spleen donor strain did not arise, although reactivity against a third party strain was detectable (Table 1).

Furthermore, co-cultured thymus cells did not suppress the response of adult C57BL/6 spleen cells against CBA/H, the tolerated strain, when added in optimal proportions according to the assay of Rich and Rich¹⁰ (Table 1) (except for a low level of suppression in one experiment). Therefore it is unlikely that the generation of a population of suppressor cells in allogeneic co-culture accounts for the lack of MLC reactivity observed. Furthermore, Ig-bearing B cells have not been found in extensive surveys of cultured thymocytes and so in the absence of immunoglobulin secretion, antigen-antibody complexes are unlikely to operate in tolerance induction in this system. Tolerance was demonstrated in seven out of 19 attempts, the remainder giving equivocal results due to the lack of MLC reactivity even in control, syngeneic co-cultures. This lack of reproducibility in obtaining an MLC reaction is probably related to technical difficulties in setting up the experiments.

A more detailed analysis of the mechanisms of tolerance induction in the systems is under way. In particular we are investigating whether antigens of the spleen donor strain are present on the surface of co-cultured thymocytes as their presence may lead to reversible or irreversible blocking of subsequent MLC reactivity. As cells are washed well before the MLC assay we feel that blocking by antigen is unlikely to account for our results. In the absence of suppression, clonal deletion may be the mechanism underlying nonreactivity in this system.

When foetal thymus was cultured for 3 d before addition of allogeneic spleen fragments, MLC tolerance was not demonstrable because cells reactive against the spleen donor strain were generated, as in control cultures (Table 2). Furthermore, if the addition of spleen fragments was delayed by 1 d, tolerance was incomplete as residual MLC reactivity against the 'tolerogen' remained (Table 2). This

Table 1 MLC response of 14-d C57BL/6 foetal thymus after organ culture for 8 d in contact with mitomycin C-treated adult syngeneic or allogeneic spleen fragments

Expt	Co-culture *Tolerogen*	Stimulator	MLC			Suppression assay % Control MLC
			mean c.p.m. ± s.e.m.	Ratio	P	
(1)	C57BL spleen ^m	C57BL	237 ± 190			
		CBA/H	1,392 ± 120	5.9	< 0.01	NT
		BALB/c	1,242 ± 292	5.2	< 0.05	
	CBA/H spleen ^m	C57BL	271 ± 226			
		CBA/H	461 ± 145	1.7	> 0.1	117
		BALB/c	1,401 ± 279	5.2	< 0.05	
(2)	C57BL spleen ^m	C57BL	72 ± 22			
		CBA/H	895 ± 225	12.3	< 0.05	NT
		BALB/c	646 ± 154	8.9	< 0.05	
	CBA/H spleen ^m	C57BL	249 ± 73			
		CBA/H	323 ± 50	1.3	> 0.1	67
		BALB/c	583 ± 51	2.4	< 0.02	
(3)	C57BL spleen ^m	C57BL	40 ± 27			
		CBA/H	5,748 ± 1,067	142	< 0.01	NT
		BALB/c	1,026 ± 214	25.4	< 0.02	
	CBA/H spleen ^m	C57BL	58 ± 75			
		CBA/H	94 ± 77	1.7	> 0.1	112
		BALB/c	690 ± 42	11.5	< 0.01	
(4)	C57BL spleen ^m	C57BL	1,818 ± 202			
		BALB/c	6,419 ± 1,012	3.5	< 0.05	NT
		CBA/H	6,824 ± 94	3.7	< 0.01	
	BALB/c spleen ^m	C57BL	2,520 ± 792			
		BALB/c	1,902 ± 348	0.8	> 0.1	95
		CBA/H	9,371 ± 1,044	3.7	< 0.01	

NT, not tested.

*1-mm³ fragments of adult spleen treated with mitomycin C (30 $\mu\text{g ml}^{-1}$) for 45 min at 37 °C and washed thoroughly. When spleen fragments were cultured alone no viable cells persisted after 3 d.

Table 2 Effect of delaying addition of spleen fragments to foetal thymus organ cultures on subsequent MLC reactivity						
Delay (d)	Co-culture		MLC		P	Suppression assay
	'Tolerogen'	Stimulator	c.p.m. ± s.e.m.	Ratio allog. syng.		
1	C57 spleen ^m	C57BL	56±27			
		CBA/H	1,960±187	34.5	< 0.001	NT
		BALB/c	1,056±75	18.6	< 0.001	
	CBA spleen ^m	C57BL	132±39			
		CBA/H	365±22	2.8	< 0.01	91
		BALB/c	1,125±263	8.5	< 0.02	
3	C57 spleen ^m	C57BL	623±137			
		CBA/H	2,927±570	4.8	< 0.02	100
		BALB/c	1,867±474	3.0	< 0.01	
	CBA spleen ^m	C57BL	701±123			
		CBA/H	2,680±184	3.8	< 0.001	124
		BALB/c	1,786±223	2.6	< 0.02	

Conditions were identical to those described in Table 1 except that spleen fragments were added to foetal thymus after a delay of 1 and 3 d, respectively.

suggests that tolerance induction depends on events occurring early in organ culture, when differentiating lymphocytes are exposed to the tolerogen but before detectable numbers of MLC reactive cells have arisen. Therefore a thymocyte precursor may be the target cell for tolerance induction. This finding parallels the recent demonstration of the tolerogenic effect of antigen on immature B cells^{10,11}. Distinct and interacting subpopulations of T cells underlie graft rejection, and unresponsiveness in one or more of these subsets may be a necessary prerequisite to allograft acceptance. In neonatal mice lymphocyte-defined and serologically-defined determinants induce unresponsiveness in precursors of MLC reactive and cytotoxic T cells respectively¹², and the former provide the major barrier to the induction of transplanatation tolerance¹³. Therefore T-cell tolerance of MLC stimulating determinants seems to be the most stringent in induction requirements.

In conclusion, our results demonstrate unresponsiveness to MLC stimulating determinants in the absence of suppression, in thymocytes generated in organ culture of foetal mouse thymus. This represents an unprecedented investigation of T-cell tolerance induced within the thymic microenvironment during ontogeny *in vitro*.

This work was supported by an MRC project grant.
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Received 28 October; accepted 9 December 1977.

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Regeneration of reduplicated limbs in
contravention of the complete circle rule

We report here a result which is in contradiction to the 'clock face model' for the regeneration of animal organs. This model^{1,2} is based on the behaviour of insect appendages, imaginal disks and the amphibian limb. It involves treating the organ as a surface on which cells are assigned positional information in terms of a radial and an angular variable. In the case of the amphibian limb, this surface corresponds to the hollow surface of limb dermis and

muscle, so that the 'radial' positional values run from the body to the tip of the digits, while the 'angular' values run around the circumference.

The model comprises two basic rules which determine whether a particular part of an organ will regenerate and which structures will be formed if it does so. The first rule is that of 'shortest intercalation' and states that when tissues of different positional values are brought together then the cells at the edges will proliferate to lay down all the intervening values. In the case of the

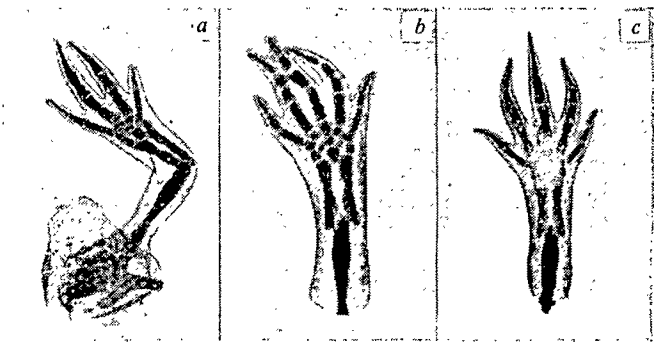


Fig. 1 a, Normal axolotl limb. $\times 9.5$; b, Reduplicated limb prepared by an operation on the tailbud embryo. $\times 12$; c, Regenerate formed after amputation of b. $\times 7.5$ (materials and methods as described in ref. 6).

angular variable, the regenerated structures correspond to the shorter of the two possible routes around the clock face. The second rule, and the one with which we are concerned here, is called the 'complete circle rule' and states that regeneration of an appendage in a distal direction (that is, away from the body) cannot take place unless a complete set of circumferential positional values is present at the cut surface. This rule has been used to explain the occurrence, position and orientation of supernumerary limbs in experiments involving the transplantation or rotation of regeneration blastemas in amphibia³.

The most spectacular evidence for the complete circle rule is an experiment performed by Bryant⁴ on double half limbs of newts. These consisted of two posterior or two anterior halves of adult forelimbs fused along the midline. They therefore possessed certain elements in two copies and lacked others altogether. Such double anterior or double posterior limbs were produced surgically, allowed to heal, and then amputated. They either did not regenerate at all, or produced only small cartilage nodules, while control combinations which possessed a complete circle of values regenerated normally.

We have carried out a closely related experiment and obtained a quite different result. We have made a number of double posterior limbs by means of grafts performed on embryos of the

axolotl *Ambystoma mexicanum*. Such limbs can be produced either by grafting a piece of flank tissue to the anterior edge of the forelimb rudiment⁵, or by grafting the central part of the limb rudiment into the flank⁶. Both operations are done at the tailbud stage before the limb bud has appeared. Figure 1(a) shows a normal axolotl forelimb and Fig. 1(b) a double posterior reduplication. Instead of a digital formula I, II, III, IV, this has IV, III, II, III, IV with corresponding elements in the wrist and arm. It lacks digit I, carpal c1, the radiale and the radius. The animals were allowed to develop for about 6 weeks by which time the reduplicated limbs are fully formed. The limbs were then amputated and a study was made of the type of structure which regenerated in their place.

Of 66 reduplicated limbs made in this way, 64 regenerated after amputation (Fig. 1c). Of these 44 gave reduplicated regenerates, not necessarily having the same number of elements as the originals, and 20 gave normal limbs, partial limbs or hybrids between normal and reduplicated limbs. The level of amputation was either the proximal upper arm (49 cases, 47 regenerates of which 30 reduplicated) or the wrist (17 cases, 17 regenerates of which 14 reduplicated). Although the original reduplications did not all contain the same number of elements they all showed a total absence of some of the normally anterior elements and must therefore have had a gap in the sequence of positional values around the circumference whatever the exact manner in which the values are assigned.

This result shows that the complete circle rule does not always apply to the amphibian limb, so whatever the reason for the failure of Bryant's limbs to regenerate, it cannot simply have been their constitution as double half limbs.

We also wish to emphasise that if the clock face model applies to embryonic development as well as to regeneration, as has been claimed³, then reduplicated limbs which lack some elements, and therefore certain of the anterior positional values, should not have been able to develop in the first place. We believe that the occurrence of reduplications in our experiments on amphibian embryos, as well as in the classical literature, can be explained by assuming that a posterior edge arises wherever embryonic limb and flank rudiments are brought into contact⁶, and that it is not necessary to invoke the clock face model to explain such phenomena.

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Received November 7 1977; accepted January 12 1978.

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Local perfusion of noradrenaline maintains visual cortical plasticity

We have formed a hypothesis which links two important, but so far separate, research areas, the monoaminergic system discovered by the Swedish group^{1,2} and the phenomenon of critical period plasticity in the visual cortex discovered by Wiesel and Hubel^{3,4}. We propose⁵ that the widespread system of monoaminergic fibres plays a part in regulating plasticity and that, more specifically, catecholamines are responsible for maintaining the high level of plasticity which is observed in the visual cortex during the critical period⁴. In an initial test of this hypothesis, we developed a dose and timing regimen of 6-hydroxydopamine (6-OHDA) to produce significant depletion of catecholamines bilaterally in the visual cortex of developing kittens⁵. The hypothesis was confirmed to the extent that

kittens treated with 6-OHDA do not have the usual cortical plasticity, as measured by a change in the ocular dominance of binocular neurones following monocular occlusion⁵. While all the results we have obtained so far with 6-OHDA are consistent with the view that catecholamines regulate cortical plasticity, other interpretations are possible because of the widespread nature of the changes accompanied by intraventricular 6-OHDA. We now present further evidence in support of the hypothesis from experiments involving microperfusion of catecholamine in localised areas of the visual cortex of animals which would not be expected to show plasticity. These experiments indicate a specific role of noradrenaline (NA) within the cortex because plastic changes are found only in the region of cortex perfused by NA while nearby cortical regions in the same kitten are unaffected.

Table 1 Ocular dominance of single neurones recorded in 11 electrode tracks in the visual cortex of seven monocularly-deprived kittens

Kittens	Ocular dominance group		
	1	2-6	7
Catecholamine-depleted cortex			
R1	4	21	6
R3 (control hemisphere)	5	23	4
R4 (control hemisphere)	11	17	8
R6 (control hemisphere)	4	25	3
*R16	2	24	7
†R5 (track 2)	2	27	5
NA-perfused cortex			
R2	7	5	22
R3 (NA hemisphere)	2	7	25
R4 (NA hemisphere)	2	8	22
R6 (NA hemisphere)	1	7	24
†R5 (track 1)	4	8	16

All kittens (except R16 and R5) had been depleted of brain catecholamine by previous intraventricular 6-OHDA in addition to the monocular deprivation.

*R16 received local perfusion of 6-OHDA (10^{-3}) alone when its right eyelid was sutured.

†R5 was perfused locally with 6-OHDA (10^{-3}) 4 d before the onset of monocular deprivation which coincided with the start of 10^{-3} NA perfusion. Details of R5 protocol are shown in Fig. 1c.

The tracks passed obliquely through the cortex and were placed symmetrically in both hemispheres of R3, R4 and R6. The control tracks in which there are high proportions of binocular neurones showed a number of ocular dominance sequences which indicated that sampling in each track tended to occur across a number of ocular dominance columns. Note that tracks through cortices which had been subjected to catecholamine depletion with local (R5, R16) or intraventricular (R3, R4, R6) 6-OHDA all yield high proportions of binocularly activated neurones (groups 2-6), despite a week of monocular deprivation to which these kittens had been subjected. In contrast, all tracks through catecholamine-depleted cortex which had been locally perfused with NA show a strong bias in favour of neurones activated exclusively by the non-deprived eye (group 7).

Twelve animals were used for this study. Seven kittens aged about 6 weeks had been depleted of cortical catecholamine, five (R1, R2, R3, R4, R6) by intraventricular treatment with 6-OHDA according to a technique already described⁵, and two (R5, R16) by local perfusion of 6-OHDA as described below for NA. A preliminary radiochemical assay⁶ of the catecholamines, dopamine and NA, in such kittens has verified the effectiveness of the intraventricular technique, which produces around 50% depletion of both catecholamines in the visual cortex. Three older animals (two aged 13 weeks, S4 and S8, and one aged 2 years, S1) were also used to test the effects of NA, for, like the 6-OHDA-treated animals, they would not be expected to show much plasticity. One normal 6-week-old kitten (S7) and one normal adult (S6) were tested by 10^{-5} NA perfusion to assess its effects upon the cortex in the absence of visual deprivation.

The right eyelid was sewn shut under ketamine and nitrous oxide anaesthesia in all animals (with the exception of S6 and S7) at the same time as cannulae were placed in the visual cortex. The cannulae were 26-gauge hypodermic needles, and

their tips were placed about 2 mm under the cortical surface with the aid of a micromanipulator and dissecting microscope. Each was cemented into the small hole which had been drilled in the skull for access and each was connected by polyethylene

tubing to an osmotic minipump (Alza) which was filled with the perfusion solution. All solutions contained 0.4% ascorbate (to limit auto-oxidation of NA) in sterile isotonic saline at pH 3. Concentrations of *L*-NA·HCl used varied from

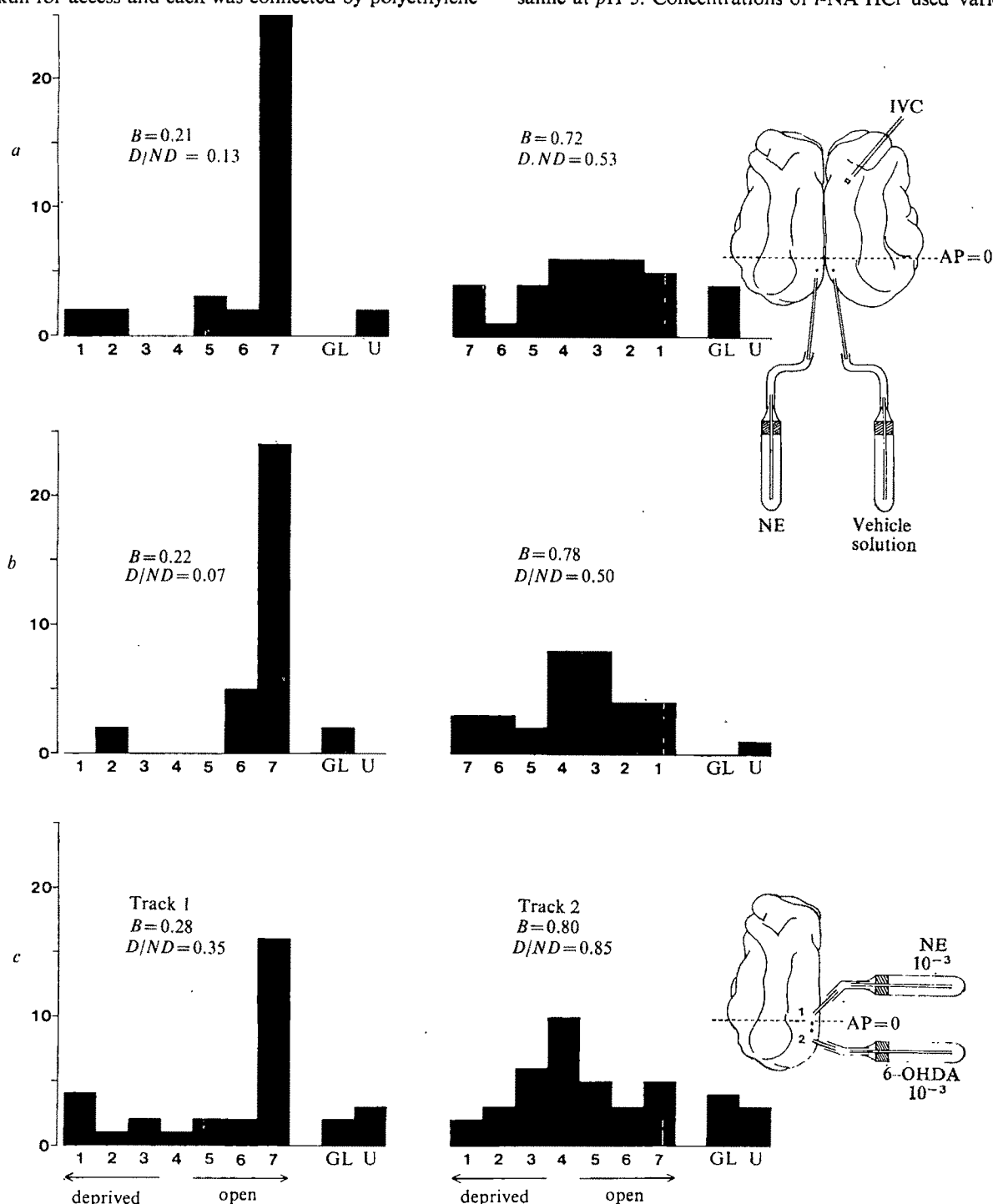


Fig. 1 Ocular dominance histograms compiled from single neurone recordings in the visual cortex of three monocularly-deprived and catecholamine-depleted kittens. They show plastic changes in regions perfused with NA (left-hand side, 10^{-4} and 10^{-5} , respectively in *a* and *b*) but not in regions perfused with the acidic vehicle solution alone (right-hand side). Ocular dominance groupings are based on the criteria of Hubel and Wiesel¹⁰. GL and U refer to lateral geniculate fibres and unresponsive units, respectively. *B* (Binocularity) is defined as the ratio of group 2-6 cells to the total number of visually responsive cells. *D/ND* (Deprived/Non-deprived) is the ratio of the number of cells dominated by the deprived eye to the number dominated by the non-deprived eye, excluding group 4 cells. Values of this index < 1 indicate a shift toward the open eye. In the diagram on the right, IVC indicates the position of the intraventricular cannula used for previous administration of 6-hydroxydopamine (6-OHDA). *a* and *b*: both kittens (R3 and R6, respectively) were treated with 6-OHDA during the sixth week to bring about bilateral catecholamine depletion according to the dose regimen reported previously³. During the seventh week (age 46 d, *a*; 48 d, *b*), right eyelid suture was performed simultaneously with the insertion of minipump/cannula combinations containing NA and vehicle solution (left hemisphere) and vehicle solution alone (right hemisphere). Seven days later, the minipump/cannula combinations were removed and single neurone recording was carried out in each hemisphere close to the site of cannulation. Note that a change of binocularity to favour the non-deprived eye occurs in cortical regions reached by NA but not in the region depleted of catecholamine and locally perfused by acidic vehicle solution alone. *c* (R5): catecholamine depletion was produced locally by microperfusion of 6-OHDA (10^{-3} concentration) from a minipump/cannula combination which was placed at the age of 48 d. Four days later, a second minipump/cannula was placed, containing 10^{-4} NA and simultaneously the right eye was closed. A further 7 d later (at the age of 60 d), single unit recording was carried out from two electrode tracks with their entry points separated by 2 mm. Only the electrode track passing through the NA-perfused region shows an ocular dominance shift. Data from an additional four kittens confirm these results and are shown in Table 1.

5×10^{-3} ($5 \mu\text{g } \mu\text{l}^{-1}$) to 10^{-5} ($10 \text{ ng } \mu\text{l}^{-1}$) in different animals. In most animals a control cannula containing the acidic vehicle solution was placed in the right visual cortex in addition to the cannula for perfusion of NA in the left visual cortex. In two cases (R5, Fig. 1c and R16) a minipump/cannula was filled with 10^{-3} 6-OHDA in the vehicle solution and placed 4 d in advance of right eye closure and the placement of a second minipump/cannula which contained either 10^{-3} NA (R5) or the vehicle solution alone (R16). After the pumps had been wetted by placement in the subcutaneous tissue of the neck, they delivered their contents at approximately $1 \mu\text{l h}^{-1}$ for one week, by which time they were empty (total capacity, $170 \mu\text{l}$). The duration of lid closure was also confined to one week synchronous with NA perfusion, at the end of which each animal was prepared for single unit recording by standard techniques⁷. Recordings were carried out in cortical area 17 with tungsten-in-glass microelectrodes⁸. Long penetrations (3–6 mm) were made by passing obliquely (5° medial and 5° anterior) down the medial bank of the postlateral gyrus to maximise the number of laminar and ocular dominance boundaries which were crossed. Symmetrical penetrations were usually made in both hemispheres. For each neurone encountered, receptive field characteristics were determined, and each was assigned to one of seven ocular dominance groups according to the criteria of Hubel and Wiesel⁹. Electrode tracks were reconstructed histologically. Sections were stained both with cresyl violet to show Nissl substance and with a silver stain for fibres. These sections showed that microelectrode tracks could pass through histologically normal cortex as close as 1 mm from the cannula tip.

As previously described, penetrations through the cortex of the 6-OHDA-treated kittens revealed a high proportion of binocular neurones despite the week-long monocular deprivation which normally produces a complete ocular dominance shift in kittens of this age. This was also true for control penetrations through regions of catecholamine-depleted cortex which had been perfused with the acidic vehicle solution alone.

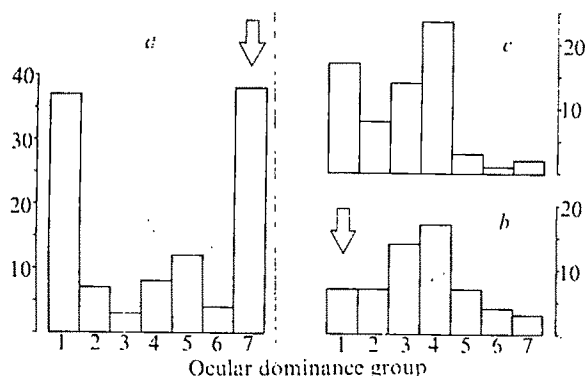


Fig. 2 Effect of NA perfusion in the visual cortex of cats which had not been subjected to previous catecholamine depletion. Conventions as in Fig. 1. Open arrow indicates open eye during period of monocular deprivation. *a*, Decreased binocularity following one week of monocular deprivation in three older cats (S1, 2 yr; S4 and S8, 13 wk). The histogram is based upon data from three electrode tracks, one from the NA-perfused hemisphere of each cat. Each electrode track yielded comparable numbers of neurones and the same ocular dominance profile. Binocularity in the three cats was respectively, S1, 30%; S4, 33%; S8, 24%. *b*, Normal binocularity obtained from one long electrode penetration in the control hemisphere (not perfused with NA) of each of the monocularly deprived cats S4 (30 neurones) and S8 (29 neurones), which showed reduced binocularity in their NA-perfused hemispheres (*a*). As expected on the basis of their age, there are no obvious changes in ocular dominance following one week's eye occlusion. *c*, Relatively normal ocular dominance shown by two cats (S7, 6 weeks; S6, > 1 yr) in which the visual cortex had been perfused with NA (10^{-3}) for one week, during which time they had normal binocular experience. The histograms from both animals were closely similar and each had the slight reduction of neurones driven by the ipsilateral eye which appears in the combined histogram.

Such penetrations were remarkable for their normality with respect to all receptive field properties of the single neurones encountered, including binocularity, which showed a normal distribution unaffected by the monocular deprivation (Fig. 1a, b; Table 1). These penetrations also confirm that our tracks adequately sampled across ocular dominance regions.

In striking contrast to the control tracks, those through cortical regions perfused with NA yielded few binocular neurones, and the ocular dominance distribution was markedly skewed in favour of the non-deprived eye (Fig. 1a, b; Table 1). All of these neurones, except for their ocular dominance bias, also appeared normal in their responsiveness and in their selectivity for stimulus attributes like orientation and direction of movement. This effect was consistent throughout all 6-OHDA-treated kittens locally perfused with NA, at concentrations from 5×10^{-3} down to 10^{-5} NA.

The presence of both normal and shifted ocular dominance distributions in different hemispheres of the same kitten provides a control for possible nonspecific effects of catecholamine depletion following 6-OHDA treatment. A localised effect is further suggested by results of the experiment illustrated in Fig. 1c, where catecholamine-depleted and catecholamine-rich cortical regions were examined side by side in the same hemisphere. The catecholamine-depleted region, treated with local 6-OHDA 4 d before the onset of monocular occlusion and NA perfusion, shows normal binocularity, while the NA-perfused cortex, only 2 mm away, shows a clear-cut ocular dominance shift.

In addition to the apparent restoration of plasticity in catecholamine-depleted kittens, NA apparently restored some degree of plasticity to the three normal, older animals. The NA-perfused cortex of all three animals yielded similar ocular dominance histograms, which showed a significant reduction in binocularity following a week-long monocular closure (Fig. 2a). These changes were restricted to the NA-perfused cortical hemisphere and were in marked contrast to the normal distributions obtained in the opposite hemisphere (Fig. 2b). No reduction in binocularity was observed in the cortex of a normal kitten and a normal adult perfused with NA (Fig. 2c), so this effect can be attributed to the abnormal visual experience with NA playing a permissive role. The change in ocular dominance seems to be particularly significant as it was produced by only one week's monocular deprivation in animals outside of the accepted limits of the critical period.

These findings therefore strengthen our hypothesis that catecholamines play a part in the regulation of critical period plasticity. We do not yet know the effects on cortical plasticity of local perfusion of other monoamines, such as dopamine and serotonin, nor do we know the type of receptor involved, although the current paradigm lends itself quite readily to answering such questions.

This work was supported by grants from the Spencer Foundation and the US PHS. We thank Marylouise Ary for help with the later experiments, Sarah Kennedy for help with histology, and Lederle Laboratories for Flaxedil.

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Received 8 July; accepted 16 December 1977.

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Connectivity changes in a class of motoneurone during the development of a nematode

THE ventral nerve cord of the nematode *Caenorhabditis elegans* contains a linear array of motoneurons (Fig. 1) which innervate the body muscles that mediate locomotion. The adult ventral cord has about three times as many cells as that of the first stage larva. The development events that generate the adult complement of cells occur in a period preceding the first larval moult. During this period we find that a class of pre-existing, juvenile motoneurons changes its pattern of connectivity. Neuromuscular junctions are removed from ventral muscles and are reformed onto dorsal muscles. Similarly the dendritic input to these neurons changes over from the dorsal to the ventral side.

The structure and connectivity of ventral cord motoneurons in adult hermaphrodites has been determined by serial section reconstructions of electron micrographs¹. The salient features of the structure are summarised below:

Motoneurons in the ventral cord can be categorised into five distinct morphological classes designated A, B, C, AS and D (Fig. 2). Classes A, B and D can be subdivided into those members that innervate ventral muscles (VA, VB and VD) and those that innervate dorsal muscles (DA, DB and DD). The members of a class are evenly distributed along the length of the cord such that all body muscles receive innervation from at least one member from each class. All motoneurons, except those in class D, receive their synaptic input from interneurons which have processes that run along the length of the ventral cord. Class D neurons, on the other hand, receive their innervation from the other classes of motoneurons. In the adult each DD motoneurone has a process which runs on the ventral side where it receives synaptic input from classes VA, VB and C motoneurons (Fig. 2). These connections are often made onto small dendritic spines which intercept neuromuscular junctions (Fig. 3a). The cell body is situated near the posterior end of the process and a branch comes off the anterior end. This branch leaves the ventral cord and runs round to the dorsal cord as a circumferential commissure. When it enters the dorsal cord it splits and runs both anteriorly and posteriorly, the posterior branch being longer. Many neuromuscular junctions (NMJs) are formed on this dorsal process (Fig. 3b) little, if any, synaptic input being received on it from the other neurone classes. The extent of both the dorsal and ventral cord processes is limited, each generally ending abruptly in a gap junction to the process of a neighbouring DD neurone. Thus the DD neurons receive their synaptic input from motoneurons

in a well defined region on the ventral side and synapse onto muscles in the same region on the dorsal side. The VD neurons have a similar shape to the DD neurons although their processes are shorter (Fig. 2). These neurons are the converse of DD neurons, receiving their innervation from motoneurons on the dorsal side and forming NMJs on the ventral side.

When the first stage larva hatches there are a total of 22 motoneurons in the ventral cord and associated ganglia. This number increases to 76 in a period of post-embryonic development that commences about 9 h before the first larval moult^{2,3}. It was found that all the VA, VB, C, AS and

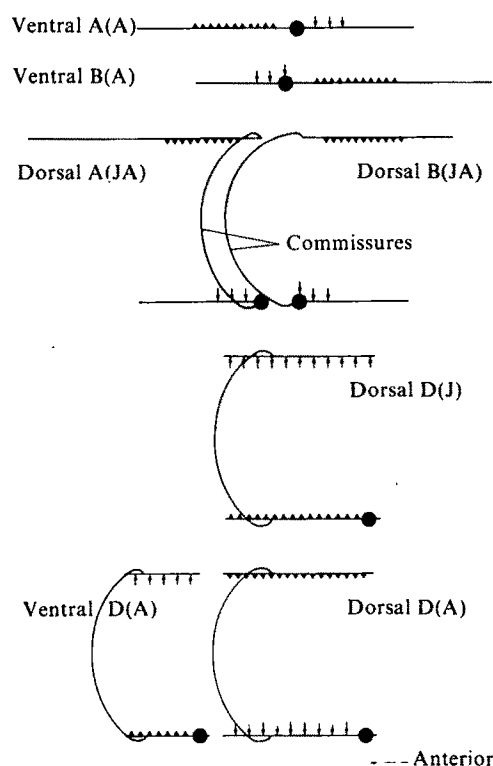


Fig. 2 Classes of motoneurone in the ventral cord. All the motoneurons of the ventral cord have a simple well defined shape. Neuromuscular junctions (▲▲▲) are made 'en passant' along the length of a process. Similarly synaptic input occurs along other processes (↑↑↑). Muscle cells send out arms to the region of the cord where the motor neurone axons reside. All motoneurons which innervate the body muscles have cell bodies (●) which are situated in the ventral cord. Each motoneurone which innervates dorsal muscles sends out a commissure which leaves the ventral cord and runs circumferentially around the animal to the dorsal side. When these processes reach the dorsal midline they turn and together make up the dorsal nerve cord. There are five distinct classes of motoneurone in the ventral cord, A, B, C, AS and D, three of which are shown. Each class has a unique pattern of synaptic input¹. Some classes are only present in adults (A), others are present in both juveniles and adult animals (J and A). Neurones in class A have axons that project anteriorly, whereas those in class B have axons that project posteriorly. Both these classes can be subdivided into neurones that innervate ventral muscles and those that innervate dorsal muscles. Class D also has both dorsal and ventral members. Neurones in this class do not receive any synaptic input from interneurons but rather from other classes of motoneurons. Ventral class D neurones receive synaptic inputs from class A, B and AS neurones on the dorsal side and innervate ventral muscles. Dorsal class D neurones in the adult (A) innervate dorsal muscles and receive their synaptic input from class A, B and C neurones on the ventral side. In the juvenile (J) they innervate ventral muscles and receive their synaptic input from DA and DB neurones on the dorsal side. Both the dorsal and ventral processes of the type D neurones end abruptly in gap junctions to the processes of neighbouring class D neurones.

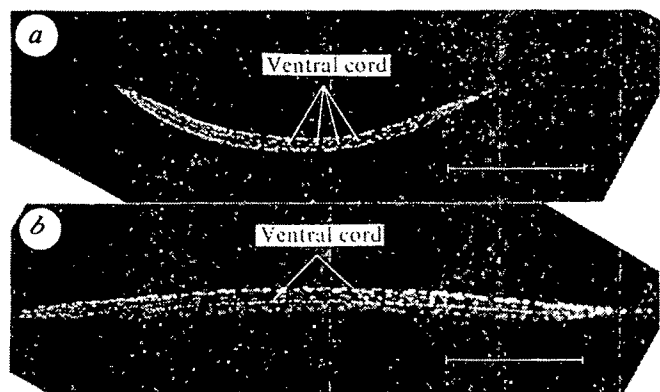


Fig. 1 Cell bodies in the ventral cords of first and second stage larvae. The number of motoneurons in the ventral cord increases in a period of post-embryonic development that commences about 9 h before the first larval moult. *a*, A first stage larva before this period has started; *b*, a second stage larva that has completed all its ventral cord cell divisions. Animals were fixed in Carnoy's and stained with the fluorescent nuclear stain Hoechst 33258. Scale bars, 100 μ m.

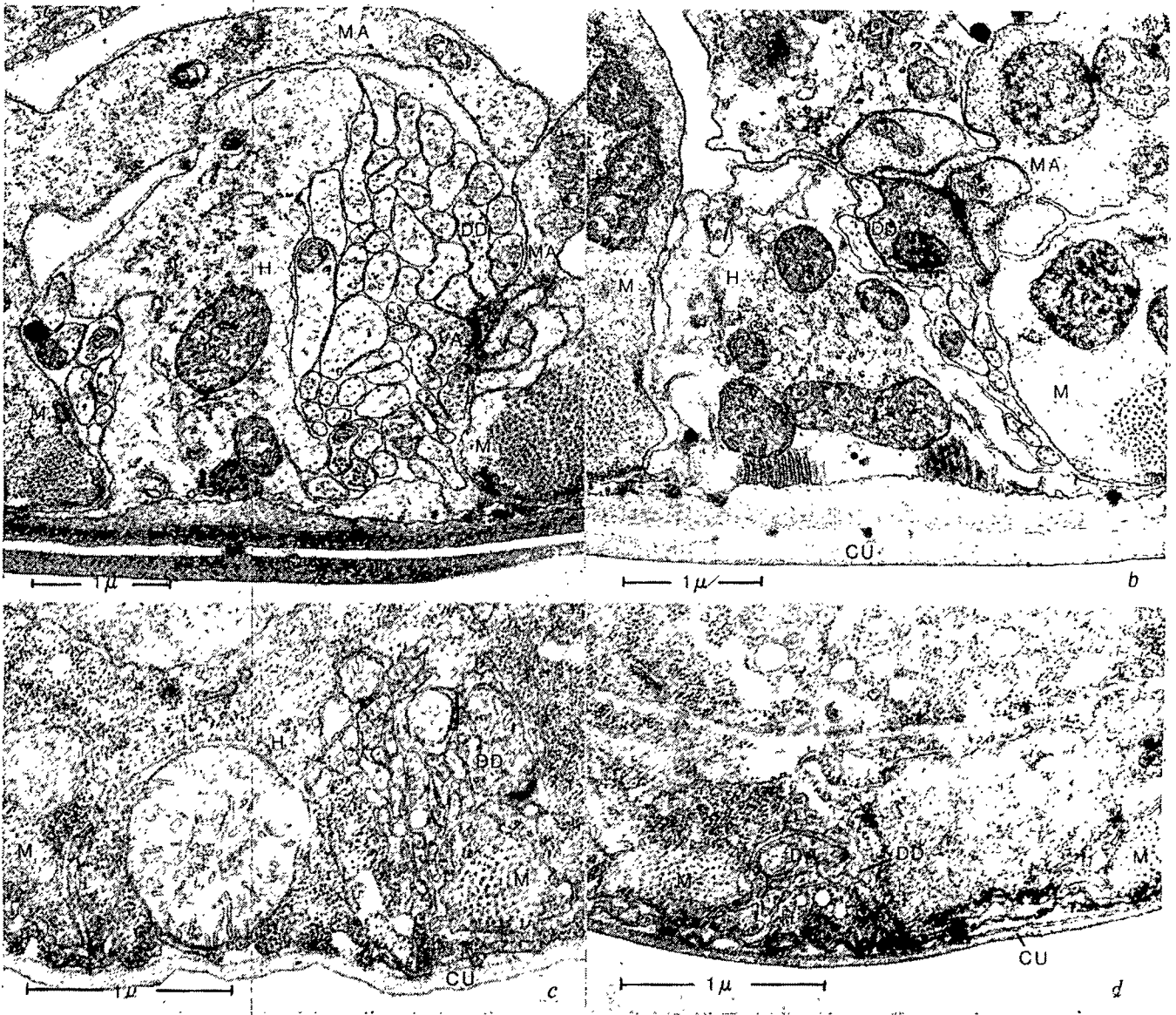


Fig. 3 Interneurons which come from the nerve ring send processes which run alongside a hypodermal ridge (H) which underlies the cuticle (CU). These interneurons synapse onto class A, B and AS motoneurons. The body muscles (M) send out arms (MA) which go to the endplate region of the motoneurone to receive their synaptic input. *a*, A VA motoneurone in the ventral cord of an adult hermaphrodite synapsing onto some muscle arms, the synapse being intercepted by the dendritic spine of a dorsal type D neurone (DD). *b*, The same DD neurone synapsing onto muscle arms in the dorsal cord (the view has been rotated 180°). There are far fewer processes in the dorsal cord as all the interneurons run in the ventral cord. *c*, A DD neurone synapsing onto muscle arms in the ventral cord of a first stage (L1) larva. This provides the sole synaptic input for the ventral side in the L1 stage yet these synapses disappear completely by the second (L2) stage. *d*, The same DD cell receiving its synaptic input on the dorsal side from a DA motoneurone. There are two classes of motoneurone which synapse onto dorsal muscles and DD cells at this stage, dorsal type A (DA) and dorsal type B (DB). Scale bars, 1 μ m.

VD neurones present in the adult are generated in this period². We deduced by subtracting these neurones from the total adult complement that juveniles must have only DA, DB and DD motoneurons in their ventral cords. This seemed paradoxical as the lack of motoneurons on the ventral side would imply that neither the ventral muscles nor the DD neurones receive any innervation.

To resolve this paradox two first stage larvae each about 10 h old were fixed, sectioned and reconstructed as described in ref. 4. Motoneurons in the juvenile cord could be unambiguously related to their counterparts in adults because of the low level of variability in the sequences of cell types along the cord^{1,2}. Cells which are DA and DB neurones in the adult were found to be the same in the larvae. This was not the case with the DD neurones, however; the morphology of the processes was the same but the

Table 1 Disposition of neuromuscular junctions in *C. elegans* larvae

		Dorsal NMJs	Ventral NMJs
First stage larva <i>a</i>	DD 1	0	20
First stage larva <i>b</i>	DD 1	0	14
	DD 2	0	15
	DD 3	0	9
Adult	DD 1	31	0
	DD 2	28	0
	DD 3	20	0

Two first stage larvae were reconstructed, *a* and *b*. The first reconstruction (*a*) covered the first DD motoneurone, the second (*b*) was longer and covered the first three DD neurones in the anterior ventral and dorsal nerve cords. The number and location of NMJs formed by these neurones is shown and compared with their counterparts in a reconstructed adult.

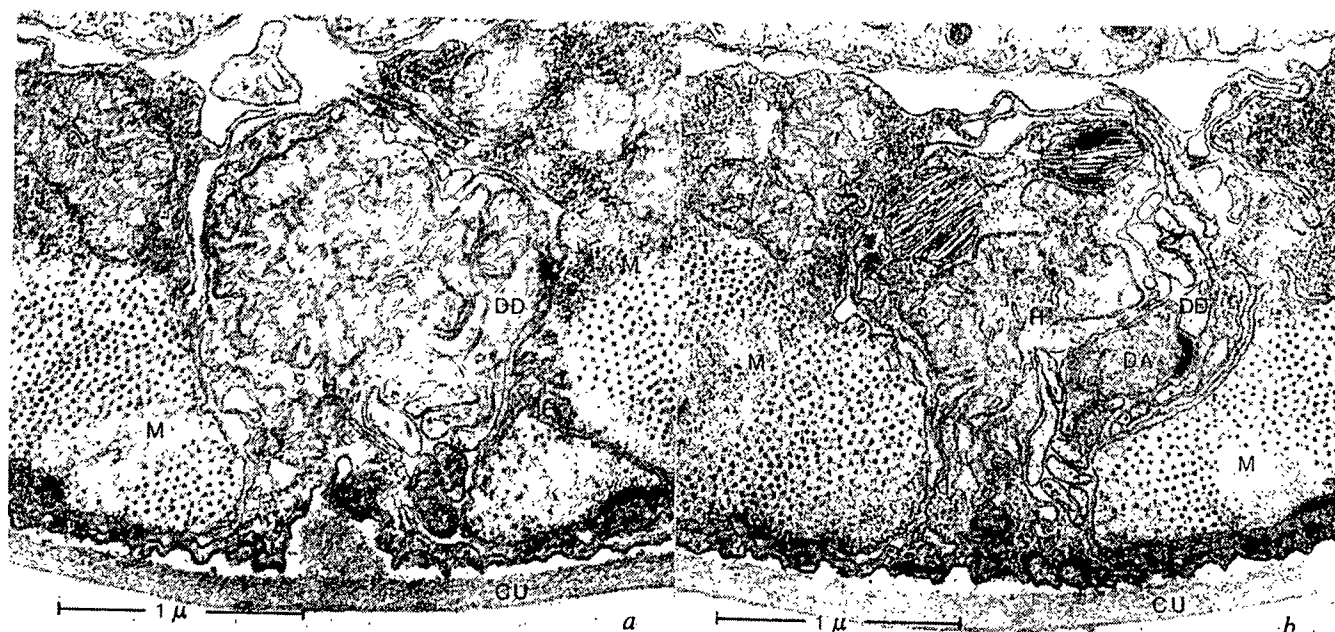


Fig. 4 *a*, The dorsal cord of a fourth stage larva of the mutant *el466*. This mutant is defective in post-embryonic development and none of the late developing motoneurones are formed. The DD neurone is synapsing onto muscles on the dorsal side with no synapses on the ventral side as in wild-type adult animals. Some of the original synaptic input to the DD neurones (that is, from the other motoneurone classes on the dorsal side) seems to persist (*b*), a feature which is seen rarely in wild-type adults. Scale bars 1 μ m.

disposition of the synapses was different (Table 1). In juveniles DD neurones had neuromuscular junctions on their ventral processes (Figs 2 and 3c) whilst their dorsal processes received synaptic input from DA and DB motoneurones (Fig. 3d) resembling the VD neurones of adults. Thus during the course of post-embryonic development all the ventral NMJ's from the DD cells disappear and new NMJ's are formed on the dorsal side. Similarly the synaptic input changes from the DA and DB neurones on the dorsal side to inputs from the newly formed VA, VB and C motoneurones on the ventral side. A late second stage larva has also been partially reconstructed and the DD neurones of this animal were found to be the same as those of the adult. It seems likely that the change in the connectivity of the DD motoneurones takes place between the first and second larval stages at the same time as the late developing motoneurones are formed^{2,3}.

Mutants have been isolated which are defective in various aspects of post-embryonic development (H. R. Horvitz, and J. E. Sulston, unpublished). In one of these mutants, *lin-6 1* (*el466*) none of the late-developing motoneurones are produced. The ventral cord was reconstructed in a fourth stage larva of this mutant and we found that the DD motoneurones formed NMJs on the dorsal side (Fig. 4a) as they do in wild-life adult animals. The DD neurones of the mutant received no synaptic input on the ventral side because the late developing motoneurones which provide all the synaptic input in wild-type adults were absent. It therefore seems likely that the DD motoneurones displace their NMJs from the ventral to the dorsal side in this mutant as they do in wild-type animals leaving the ventral muscles with no synaptic input. This is consistent with the observation that the first stage larvae of this mutant have apparently normal locomotory behaviour, whereas all the later stages are uncoordinated in their body movements (J. E. Sulston, personal communication). There are some indications the DD neurones of the mutant continue to receive some synaptic input from DA and DB neurones on the dorsal side (Fig. 4b) unlike wild-type adults that rarely have such connections¹. The observations that the DD neurones in *el466* change those sites where NMJs are formed in the same way as those in wild-type animals suggest that this

process is independent of the formation of NMJs from the late developing neurones. This may not be the case for the sites of synaptic input for the DD neurones since some of the juvenile sites seem to persist in this mutant.

Connectivity changes in normal⁴ and abnormal development⁵ have been described in other organisms. The behaviour of the DD neurones in *C. elegans* provides a rather extreme example of neuronal plasticity during development where not only are connections altered but also the direction of information flow in processes is reversed.

We thank Nicol Thomson for technical assistance and Sydney Brenner, Bob Horvitz and John Sulston for helpful discussions.

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Received 8 August; accepted 16 December 1977.

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Dopamine receptors localised on cerebral cortical afferents to rat corpus striatum

STRIATAL dopamine receptors, monitored by dopamine-sensitive adenylate cyclase activity^{1,2} or binding of ³H-haloperidol³⁻⁵ apparently represent distinct entities because of differences in drug sensitivity and the pattern of their ontogenetic development^{6,7}. Dopamine also elicits both excitatory and inhibitory effects on striatal neurones^{8,9}. We have examined the effects of selective degeneration of striatal intrinsic neurones with the neurotoxin, kainic acid^{10,12}, and elimination of cortico-striate afferents by cortical ablation¹¹ on the dopamine receptors in rat striatum.

We present evidence that a substantial portion of striatal ^3H -haloperidol receptor sites are localised to axons of cerebral cortical afferents whereas dopamine-sensitive adenylate cyclase is confined to neurones intrinsic to the striatum.

After anaesthesia with Equi-thesin (0.6 ml, Jensen-Salisbury), Sprague Dawley rats (160 g) were positioned in a David Kopf small animal stereotaxic apparatus and a 0.3-mm Hamilton cannula, was inserted into the striatum through a burr hole in the calvarium (coordinates: 7.9 A; 2.6 L; 4.8 V). Kainic acid (Sigma) 2 μg in 1 μl of artificial cerebrospinal fluid titrated to pH 7.4, was infused and the scalp was then apposed with sutures. For unilateral cortical ablations, the calvarium overlying the parietal and frontal cortex was removed and the underlying cortex was removed to the level of the corpus callosum by aspiration or by shallow knife cuts; bleeding was controlled with gel foam. The rats received a prophylactic intraperitoneal injection of ampicillin. Ten days after a striatal kainate injection, the cortex on the lesioned side was ablated for the studies of additivity. At various times after kainic acid injection or 5 d after cortical ablation, the rats were decapitated and the corpus striatum ipsilateral to the lesion, the contralateral unlesioned striatum and striata from unlesioned animals were assayed for ^3H -haloperidol binding³, dopamine-sensitive adenylate cyclase, glutamic acid decarboxylase, choline acetyltransferase and tyrosine hydroxylase as previously described¹².

Twenty-four hours after striatal kainate injections, ^3H -haloperidol binding and dopamine-sensitive adenylate cyclase were not significantly different from control values; however, by 2 d after injection, dopamine-sensitive adenylate cyclase was reduced by 85% compared with contralateral and control values whereas ^3H -haloperidol binding was reduced by only 20% (Fig. 1a). The 85% decline in dopamine-sensitive adenylate cyclase was maintained for at least 22 d after kainate lesion and was associated with a similar reduction in this cyclase in the ipsilateral substantia nigra, an area innervated by striatal neurones¹⁴. ^3H -haloperidol binding fell to 60% of control levels with a maximal reduction at 6 d and no further change apparent 22 d after kainate lesion. The kinetics of ^3H -haloperidol binding were determined by Scatchard analysis (Fig. 1b). The dissociation constant for ^3H -haloperidol, 0.9 nM, was the same in control and lesioned striata; however, the maximum number of binding sites was reduced by 40% in the kainate-treated striatum. As previously reported¹², at 22 d after striatal kainate lesion, tyrosine hydroxylase activity was not significantly different from control but there was a

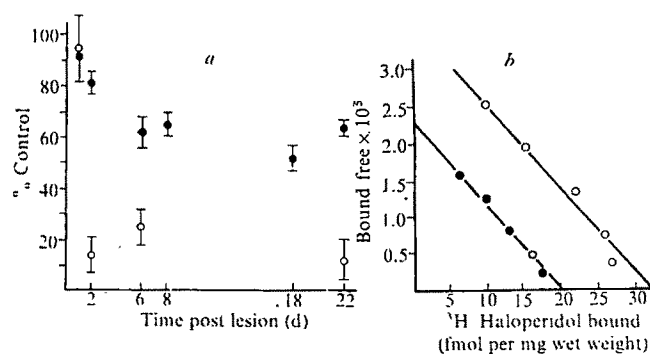


Fig. 1 a, Time course of loss of striatal dopamine-stimulated adenylate cyclase activity (○) and ^3H -haloperidol receptor binding (●) after unilateral striatal kainate injection. Adenylate cyclase activity was stimulated by 50 μM dopamine with basal levels measured in the presence of 5 μM fluphenazine; ^3H -haloperidol binding was assayed with 0.8 nM ^3H -haloperidol in the presence of absence of 0.1 mM dopamine. b, Scatchard analysis of striatal ^3H -haloperidol binding 22 d after unilateral kainate injection. Binding was measured at five concentrations of ^3H -haloperidol (0.5–8.0 nM) in the presence and absence of 0.1 mM dopamine in the lesioned (●) and contralateral control striata (○) pooled from five rats. The experiment was repeated three times and the Scatchard plots drawn from the mean data. For lesioned striata, $K_D = 0.89$ nM and B_{max} (maximum binding) = 20.4 pmol per g wet weight. In control striata, $K_D = 0.90$ nM, $B_{max} = 32.5$ pmol per g wet weight.

60% reduction in the activities of choline acetyltransferase and glutamic acid decarboxylase, enzymatic markers for the cholinergic and GABAergic neurones intrinsic to the striatum (Table 1). Thus, kainate-induced changes in dopamine-sensitive adenylate cyclase and ^3H -haloperidol binding differ both in extent and in time course.

The fact that 60% of the haloperidol binding sites remain intact after ablation of the intrinsic neurones of the striatum by kainic acid suggests that some of these sites are localised on intrastriatal axons of neurones with cell bodies lying outside the striatum. To investigate this possibility, we evaluated the effects of cortical ablation, which destroys a major projection to the striatum¹⁵. The activity of tyrosine hydroxylase, an enzyme localised to dopaminergic terminals in the striatum was used as an index of nonspecific damage after the cortical lesion; only striata in which the activity of tyrosine hydroxylase was at least 85% of control striata were used for computation of ^3H -haloperidol binding. In decorticate rats, the specific striatal binding of ^3H -haloperidol decreased by 35% (Table 1). Scatchard analysis of the kinetics of binding indicates that the reduction reflects

Table 1 Effect of kainic acid lesion and cortical ablation on rat striatal enzyme activity and ^3H -haloperidol binding

	^3H -Haloperidol binding (% control \pm s.e.m.)	Tyrosine hydroxylase activity	Choline acetyltransferase activity	Glutamic Acid decarboxylase activity
Kainate 22 d post-lesion	64 \pm 3* (6)	97 \pm 6 (6)	49 \pm 4† (6)	42 \pm 7‡ (6)
Cortical ablation 5 d post-lesion	68 \pm 5† (22)	101 \pm 3 (22)	73 \pm 5† (16)	99 \pm 7 (12)
Kainate and cortical ablation	30 \pm 5† (9)	120 \pm 9 (9)	39 \pm 6† (9)	41 \pm 6† (8)

Rats received an intrastriatal injection of kainic acid (2 μg) or cortical ablation or kainate injection followed by cortical ablation as described in the text. The striata were assayed for the activities of tyrosine hydroxylase, choline acetyltransferase and glutamate decarboxylase¹⁰ and specific binding of ^3H -haloperidol³. Results are presented as % (\pm s.e.m.) of the level in the contralateral nonlesioned striata which did not differ significantly from those in unlesioned rats. Absolute values for the contralateral striatum expressed in terms of mg tissue: ^3H haloperidol binding 0.15 fmol (710 c.p.m. per sample) tyrosine hydroxylase, 230 pmol h^{-1} ; choline acetyltransferase, 20 nmol h^{-1} ; glutamic acid decarboxylase 13 nmol h^{-1} . The number of separate preparations is indicated in parentheses.

* $P < 0.005$.

† $P < 0.001$.

‡From ref. 13.

a loss of receptor sites with no alteration in affinity for the ligand. In contrast, the dopamine stimulated formation of cyclic AMP in striatal homogenates was not significantly altered by decortication (lesioned 10.5 ± 1.5 pmol $\text{mg}^{-1} \text{min}^{-1}$, control 12.5 ± 1.0 pmol $\text{mg}^{-1} \text{min}^{-1}$; $N = 9$). Basal cyclase (16.0 ± 2.0 pmol $\text{mg}^{-1} \text{min}^{-1}$) in the decorticate striata was reduced by 37% as compared to control. The activities of tyrosine hydroxylase and glutamic acid decarboxylase were not altered by decortication but a modest but consistent 27% ($P < 0.001$) reduction in choline acetyltransferase activity is observed. Less extensive cortical lesions do not reduce striatal choline acetyltransferase^{15,16}.

In rats in which striatal kainate injection was followed by ablation of the overlying cerebral cortex, the specific binding of ³H-haloperidol decreased by 70%, indicating additive effects of the two lesions (Table 1). The activities of choline acetyltransferase and glutamic acid decarboxylase in the combined lesioned striatum were not significantly different from those in striata after kainate injection alone. Histological examination of Nissl-stained sections of striata following kainic acid lesions revealed a near complete loss of neuronal cell bodies, a gliotic reaction and intact internal capsule fibres in agreement with detailed histological studies of the lesion¹⁷. After cortical ablation, the intrinsic neurones appear normal but a gliotic reaction limited to the internal capsule fibres is evident.

These observations indicate a clear dissociation between the activity of dopamine-sensitive adenylate cyclase and ³H-haloperidol binding sites in the striatum. The nearly complete loss of the dopamine-sensitive adenylate cyclase following striatal kainate lesion indicates that it is largely confined to neurones intrinsic to the striatum and their nigral projections while the persistence of 65% of the ³H-haloperidol binding in the same striatum suggests that a majority of the binding sites are contained on other tissue elements. The additional depletion of ³H-haloperidol binding after cortical ablation favours its localisation to axons or terminals of the cerebral cortical neuronal input to the striatum. Most pharmacological effects of neuroleptics correlate better with affinity for ³H-haloperidol binding sites than with effects on the dopamine-sensitive cyclase⁸, suggesting that the dopamine receptors localised to cortical afferents may represent a major site of pharmacological action of the drugs.

This work was supported by USPHS and McKnight Foundation grants to I.C., J.T.C. and S.H.S.; we thank Robert Zaczek and Timothy Prosser for technical assistance and Susan M. Garonski for manuscript preparation.

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Coenzyme A is a purine nucleotide modulator of acetylcholine output

FUNCTIONAL roles have been proposed for the purine nucleotides and nucleosides shown to be released by several neural tissues^{1,2}. In the autonomic nervous system, ATP or a related purine compound has been ascribed a transmitter role in the 'purinergic' nerve hypothesis³. Although some metabolites of ATP have been demonstrated in perfusates of field-stimulated gastrointestinal smooth-muscle preparations⁴, the precise identity of the mediator released from 'purinergic' nerves has not been established, and the existence of such nerves is not widely accepted⁵. Structure-activity considerations suggested to us that coenzyme A (CoA), a substituted purine nucleotide, should interact with the 'purinergic' receptor—to our knowledge there are no reports of such interactions, or of any other autonomic effect of CoA, in the literature. We report here the pre-synaptic inhibitory effects of CoA and its analogues on acetylcholine release.

The effects of CoA and its analogues (acetyl-CoA, adenosine 3'5'-diphosphate (adenosine 3'5' diPO₄)) on the mechanical activity of electrically-stimulated guinea-pig ileum⁶ were compared with those of the nucleotides ATP, 3'5'-cyclic AMP, dibutyl-3'5'-cyclic AMP (dbcAMP) and to noradrenaline, pantothenic acid and β -mercaptoethylamine. CoA (4.0×10^{-7} – 5.0×10^{-6} M), acetyl-CoA (4.0×10^{-7} – 4.6×10^{-6} M), adenosine 3'5' diPO₄ (4.0×10^{-7} – 1.6×10^{-6} M) and ATP (4.0×10^{-7} – 2.5×10^{-6} M) produced concentration-dependent reductions of the response of the ileum (Fig. 1) without changing the sensitivity of the preparation to exogenous acetylcholine (ACh).

Figure 2 shows the relative potencies of these compounds, adenosine 3'5' diPO₄ being the most potent. Pantothenic acid and β -mercaptoethylamine (substituents in the side chain of CoA) were inactive at concentrations as high as 1×10^{-4} M. The inhibitory effects of CoA and its analogues depended on the frequency of stimulation, contractions induced by high frequency (5 and 10 Hz) being reduced by approximately 32% at concentrations (5×10^{-8} – 1×10^{-5} M) which produced a 100% inhibition at 0.2 and 1 Hz. Intact adrenergic mechanisms were not necessary for the inhibition; it was not altered by treatment with either phentolamine (5×10^{-6} M) plus propranolol (1×10^{-6} M) or guanethidine (1×10^{-5} M).

Theophylline, a blocker of adenosine receptors⁷⁻⁹, produced a concentration-dependent enhancement of the responses to stimulation of Auerbach's plexus. The responses were enhanced by theophylline (2.5×10^{-3} – 1×10^{-4} M), this being more pronounced at low frequencies of stimulation (0.2–1 Hz) than at high (5–10 Hz). Parallel displacements to the right by theophylline of the log concentration-response curves of CoA, acetyl-CoA, adenosine 3'5' diPO₄ and ATP (Fig. 2 a–d; P for slopes > 0.05) suggested competitive antagonism. Analysis of this antagonism by the method of Arunlakshana and Schild¹⁰ yielded linear isoboles, the slopes of which were not significantly different from unity ($P > 0.05$, Fig. 2 e–h). In addition, the apparent pA_2 values (derived from Fig. 2) were similar, indicating that these agonists and the antagonist all act at the same receptor. Antagonism by theophylline is not mediated through elevation of intracellular cyclic AMP because neither cyclic AMP (5×10^{-7} – 5×10^{-6} M) nor the dibutyl derivative (1×10^{-6} – 1×10^{-5} M) added exogenously altered the effects of CoA or the other purine nucleotide agonists.

Dipyridamole, a compound which potentiates the effects of adenosine¹¹⁻¹⁴, in concentrations up to 9.9×10^{-7} M reduced the height of the contractions of the ileum without influencing the sensitivity to added ACh (Fig. 3). Dipy-

ridamole also potentiated the inhibitory effects of CoA, adenosine 3'5'-diPO₄, and ATP, producing 48-, 34- and 49-fold decreases in the EC₅₀ values respectively (see Fig. 3). Theophylline (2.5×10^{-5} – 1×10^{-4} M) prevented the effects of dipyrindamole on the responses of the muscle strip both to stimulation at 0.2 Hz and to CoA and the other purine nucleotides.

The effects of CoA and the other purine nucleotide agonists were also studied on the resting and stimulated release of ACh from Auerbach's plexus as assayed on isolated guinea-pig ileum by the method of Paton and Vizi⁶. At concentrations of 2×10^{-6} M, these compounds significantly ($P < 0.05$) reduced the resting and stimulated (0.2 Hz) release of ACh (Table 1). The effects of these compounds were concentration-dependent, maximal inhibi-

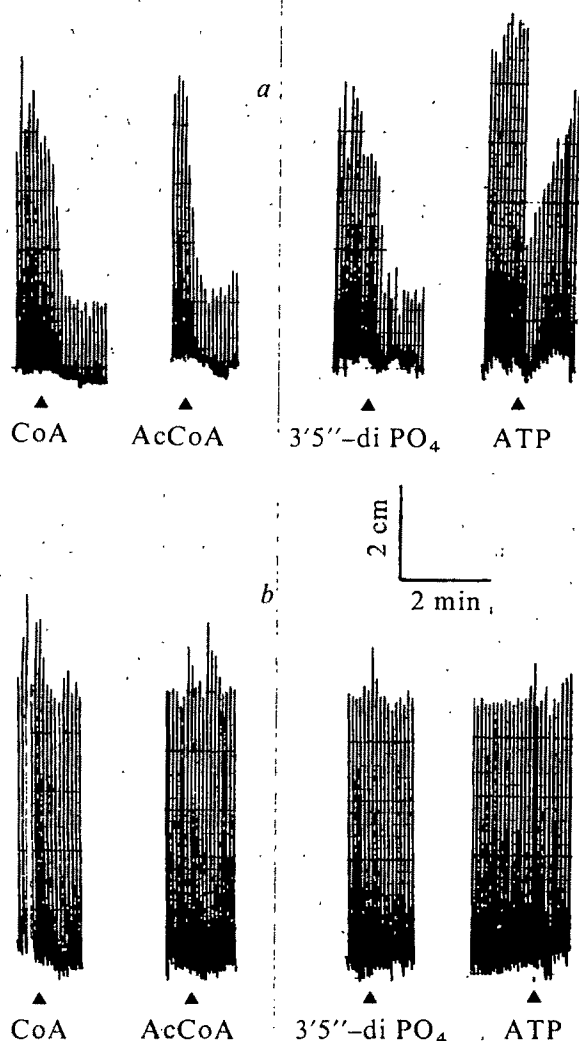


Fig. 1 Responses of electrically stimulated guinea-pig ileum to purine nucleotide agonists in the absence (a) and presence (b) of theophylline. Agonists were added at arrows. Longitudinal muscle strips from male guinea pigs (250–400 g) were prepared according to the method of Paton and Vizi⁶ and set up in 10-ml organ baths containing Krebs's solution (composition, in μ M: KCl 4.75; CaCl₂ 2.54; KH₂PO₄ 1.19; NaCl 118; NaHCO₃ 25; and glucose 11.0) and bubbled with 95% CO₂. Temperature was maintained at 37 °C and the pH at 7.3. Longitudinal contractions were recorded auxotonically with a Harvard Apparatus smooth muscle transducer using an applied load of 1 g. Field stimulation⁶ was achieved by means of trains of supramaximal rectangular pulses of 1 ms duration applied through platinum electrodes at the top and bottom of the bath. Frequency of stimulation was 0.2 Hz. Concentrations of agonists used were: coenzyme A (CoA) 3.0×10^{-6} M, acetyl-coenzyme A (AcCoA) 3.0×10^{-6} M, adenosine 3'5' diphosphate (3'5' diPO₄) 9.5×10^{-7} M, and ATP 1.7×10^{-6} M. Tissues were exposed to theophylline (5×10^{-5} M) for 30–45 min before addition of agonists in all experiments.

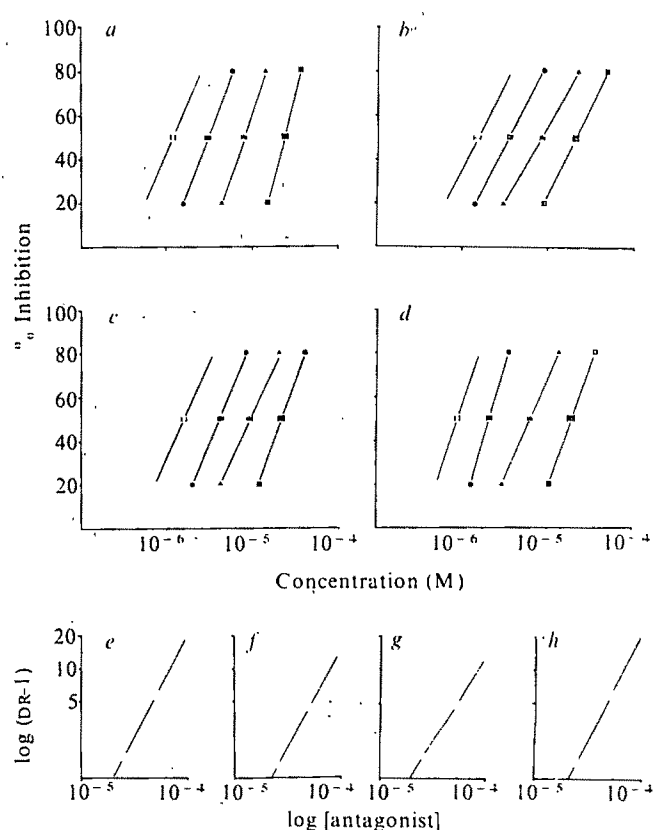


Fig. 2 a–d, Log concentration–response curves for the purine nucleotide agonists on electrically stimulated guinea pig ileum in the absence and presence of theophylline. Responses to ATP (a), CoA (b), AcCoA (c) and 3'5' diPO₄ (d) are shown in the absence (○) and presence of theophylline, 2.5×10^{-5} M (●), 5×10^{-5} M (▲) and 1×10^{-4} M (■). Responses were calculated as percentages of maximum inhibition. Concentration of agonists yielding 50% of maximum response (EC₅₀) are ATP: 1.2×10^{-6} M, CoA: 1.5×10^{-6} M, AcCoA: 1.6×10^{-6} M, and 3'5' diPO₄: 8.8×10^{-7} M. Regression lines constructed from pooled results of three to five experiments, were calculated using the least squares method and ranges of the 95% confidence intervals (bars at EC₅₀) obtained by the method of Wonnacott and Wonnacott²². e–h, Schild¹⁰ plots showing competitive nature of the antagonism between theophylline and the purine nucleotide agonists. Plots of log dose ratio – 1 (DR – 1) against log concentration of theophylline yielded apparent pA_2 values of 4.70 for ATP (a), 4.68 for CoA (b), 4.74 for AcCoA (c) and 4.67 for 3'5' diPO₄ (d). Values for slopes were not significantly different from 1 ($P > 0.05$).

tion being obtained, at concentrations of 1 to 8×10^{-5} M. No tachyphylaxis was observed. Theophylline 10^{-4} M markedly enhanced the release of ACh both at rest and following stimulation ($P < 0.05$). The inhibitory effects of CoA and its analogues, as well as of ATP, on ACh release were antagonised by theophylline (Table 1).

CoA and its analogues are thus able to reduce the release of ACh from Auerbach's plexus and theophylline competitively antagonises these inhibitory effects. The observation that theophylline enhanced both the mechanical activity and the release of ACh poses the question of whether endogenous CoA and/or its derivatives might control the release of ACh. If so, it is possible that the amount of ACh measured represents an already reduced output. This could explain why the output of ACh per stimulus is very low when a high frequency of stimulation is applied¹⁸, a situation in which greater amounts of CoA might be released to act as a purine nucleotide brake on ACh output. This same role might be invoked for the observed effects of ATP.

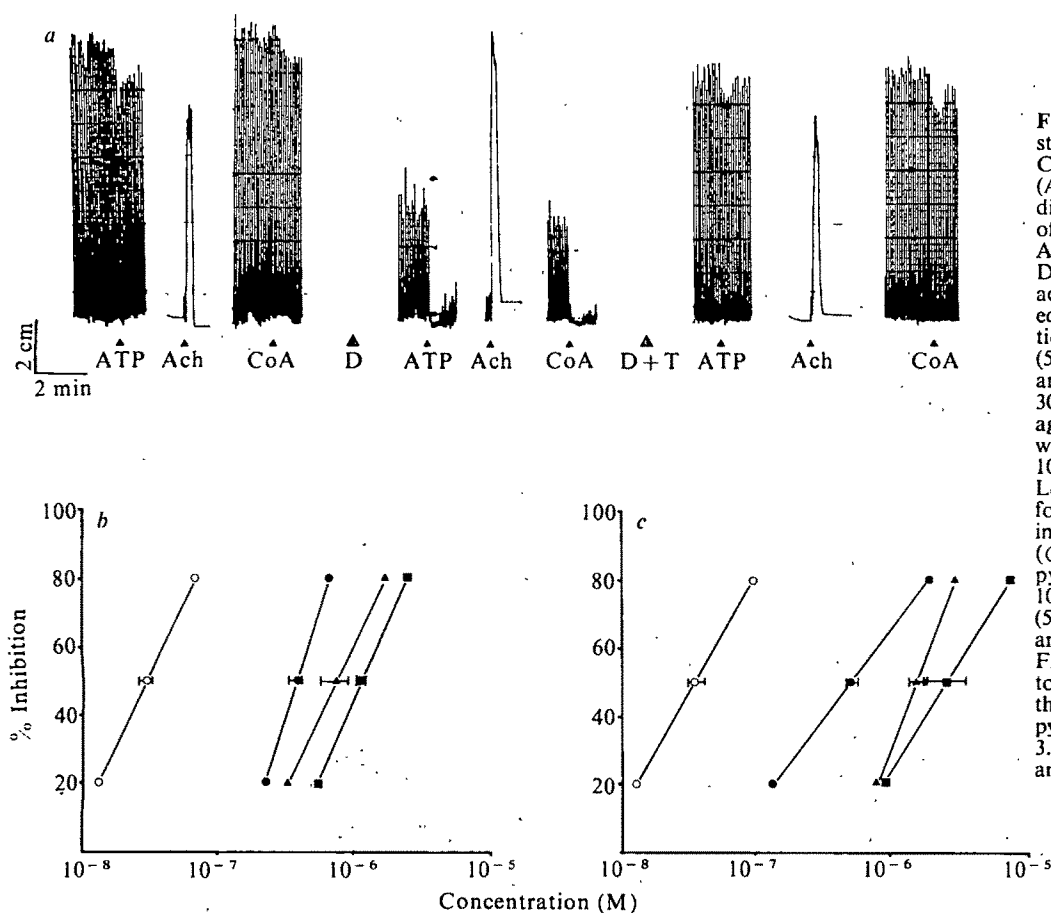


Fig. 3 *a*, Responses of electrically stimulated guinea pig ileum to CoA, ATP, and acetylcholine (ACh) alone, in the presence of dipyrindamole, and in the presence of dipyrindamole plus theophylline. Agonists were added at arrows. Dipyrindamole (9.9×10^{-7} M, D) was added at the arrow and allowed to equilibrate for 30 min before addition of agonists. Theophylline (5.0×10^{-3} M, T) was added at arrow and allowed to equilibrate for 30–45 min before addition of agonists. Agonist concentrations were CoA: 2.0×10^{-7} M, ATP 2.0×10^{-7} M and ACh 5.0×10^{-8} M. *b*, *c*, Log concentration–response curves for ATP (*b*) and CoA (*c*) alone (■) in the presence of dipyrindamole (○), and in the presence of dipyrindamole + theophylline 2.5×10^{-5} M (●) or dipyrindamole + Theo (5×10^{-5} M) (▲). Regression lines and ranges were calculated as for Fig. 2 using pooled data from three to five experiments. EC_{50} values in the absence and presence of dipyrindamole are 1.5×10^{-6} M and 3.5×10^{-6} M respectively for CoA and 1.4×10^{-6} M and 2.9×10^{-6} M respectively for ATP.

Neurally-released ATP has been detected using the firefly luciferase luminescence technique¹⁸ or has been inferred from chromatographic detection of degradation products such as inosine and hypoxanthine⁴. The neural origin of such ATP is not in question, but the exact nerves involved are³. A 'purinergic' nerve, synthesising and releasing ATP as the putative neurotransmitter, has been proposed³. Our evidence of enhanced ACh output after theophylline as well as the ability of dipyrindamole to attenuate the mechanical response, supports the notion of an endogenously-released purine-like compound which acts presynaptically to reduce ACh output from cholinergic nerve terminals. We have shown that exogenous ATP as well as CoA and its analogues do indeed inhibit the release of ACh from guinea-pig ileum. The presence on cholinergic nerve terminals, therefore, of receptors with affinity for these purine nucleotide agonists must be accepted. The same arguments which support a role for ATP functioning as a physiological modulator of the release of ACh could well apply to CoA. We have shown that in all circumstances where ATP might fulfil a modulatory role, CoA is at least as effective. The source of purine-like compounds might indeed be the 'purinergic' nerve; however, in view of the apparent difficulties in distinguishing structurally between cholinergic and 'purinergic' axons³, this does not seem likely.

CoA could fulfil the role of the endogenously-released modulator; it is present in cholinergic nerve terminals in amounts which have been claimed to be as much as ten times higher than the amount of ACh¹⁷. Even if this value is an overestimate, the nature of the reactions involved in the synthesis of ACh in cholinergic nerve terminals dictates that there should be at least equimolecular quantities of CoA and ACh. Choline acetyltransferase (ChAT, EC 2.3.1.6) utilises choline and acetyl-CoA as its substrates in a bi-ordered Theorell–Chance type of reaction^{18,19} to yield ACh and CoA as products. The concentrations of CoA

in the cytosol of cholinergic nerve terminals must not only match the ACh concentration but, since the same extra-mitochondrial pool of CoA is the precursor of the substrate Acetyl-CoA, and since this substrate has been shown not

Table 1 Effect of purine nucleotides on ACh output of resting and electrically stimulated guinea-pig ileum in the absence and presence of theophylline

Agonist	ACh output ($\text{mol} \times 10^{-12} \text{ g}^{-1} \text{ min}^{-1}$)	
	Resting	Stimulated (0.2 Hz, 1 ms)
Control	25.9 ± 1.0	73.3 ± 3.2
Control + Theo (100 μM)	34.6 ± 1.2 (+33.5%)*	85.8 ± 2.8 (+14.6%)
ATP (2.0×10^{-6} M)	13.9 ± 0.9 (–46.3%)	20.8 ± 0.9 (–71.6%)
ATP + Theo.	26.2 ± 3.3 (+1.2%)	39.4 ± 0.9 (–46.2%)
AcCoA (2.0×10^{-6} M)	15.0 ± 2.0 (–42.1%)	40.8 ± 1.6 (–44.3%)
AcCoA + Theo.	27.4 ± 3.4 (+5.8%)	63.5 ± 1.7 (–13.4%)
CoA (2.0×10^{-6} M)	10.4 ± 1.1 (–59.8%)	33.4 ± 1.4 (–54.4%)
CoA + Theo	25.6 ± 3.3 (–1.2%)	56.1 ± 2.3 (–23.4%)
3'5' diPO ₄ (2.0×10^{-6} M)	13.1 ± 2.5 (–49.4%)	41.4 ± 5.2 (–43.5%)
3'5' diPO ₄ + Theo	23.7 ± 3.1 (–8.5%)	57.8 ± 3.9 (–21.1%)

Acetylcholine output was assayed according to Paton and Vizi⁹ with modifications. Physostigmine sulphate (5.0×10^{-6} M) was present in the 10-ml test bath to prevent hydrolysis of ACh. Morphine sulphate (1.5×10^{-5} M) and physostigmine sulphate (2.5×10^{-9} M) were present in the 10-ml assay bath to reduce endogenous ACh release and to increase the sensitivity of the assay preparation to ACh. Responses to standard solutions of ACh containing the final concentrations of the agonists used in the test bath were obtained on the assay preparation and standard curves established. Aliquots of 0.2–0.5 ml from the test bath were removed following 30-min test periods for either resting or stimulated conditions and assayed on the assay preparation. ACh outputs in stimulated conditions are expressed as the total stimulated output minus the appropriate resting output.

*Results, expressed as $\text{mol} \times 10^{-12} \text{ g}^{-1} \text{ min}^{-1} \pm \text{s.e.m.}$, were calculated from the pooled results from three to five experiments. Percentage increases (+) or decreases (–) in ACh output were calculated from the appropriate control output.

to be the rate-limiting substrate for the ChAT reaction²⁰, the CoA concentration may, in fact, be greater than the ACh concentration. Were CoA (or a derivative) to be released from cholinergic nerve terminals, it would not be detected by the firefly luciferase method because that enzyme has a high degree of specificity for ATP. The amount, therefore, of purine-like compound released on stimulation may have been greatly underestimated. In addition, the same breakdown products which allowed the inference of ATP release¹ to be made would result from the degradation of CoA.

If CoA (or any other purine nucleotide) were to be released from cholinergic nerve terminals on stimulation and function as an inhibitory modulator, the production of either an excitatory or an inhibitory junction potential at the muscle would result depending on the balance between the concentrations of ACh and the inhibitory modulator. Such a balance could be dependent on the frequency of stimulation, the distribution of receptors or differential rates of breakdown or uptake of the two mediators. This concept might be useful in explaining the occurrence of both excitatory and inhibitory junction potentials following stimulation of non-adrenergic inhibitory nerves^{5,21}.

These results do not demand that CoA itself be the endogenous modulator. In fact, our studies showed that adenosine 3'5'-diPO₄, a compound likely to result from the breakdown of CoA, was more potent than CoA itself. Equally, it is possible that the compound which represents the penultimate step in the synthesis of CoA, dephospho-CoA (preliminary studies have shown activity equal to CoA), could act in this role.

This study raises the distinct possibility that CoA, released from cholinergic nerve terminals may function as the modulator of ACh release—a purine nucleotide brake and suggests that the 'purinergic' nerve hypothesis of Burnstock³ as well as some of the characteristics of cholinergic nerve transmission should be re-examined.

This study was supported by the MRC of Canada. We thank Miss M. Ackerman for technical assistance and Dr C. W. Gowdey for helpful discussions. F.K.O. is the recipient of a Mid-West Nigerian Government Scholarship.

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Received 18 August; accepted 12 December 1977.

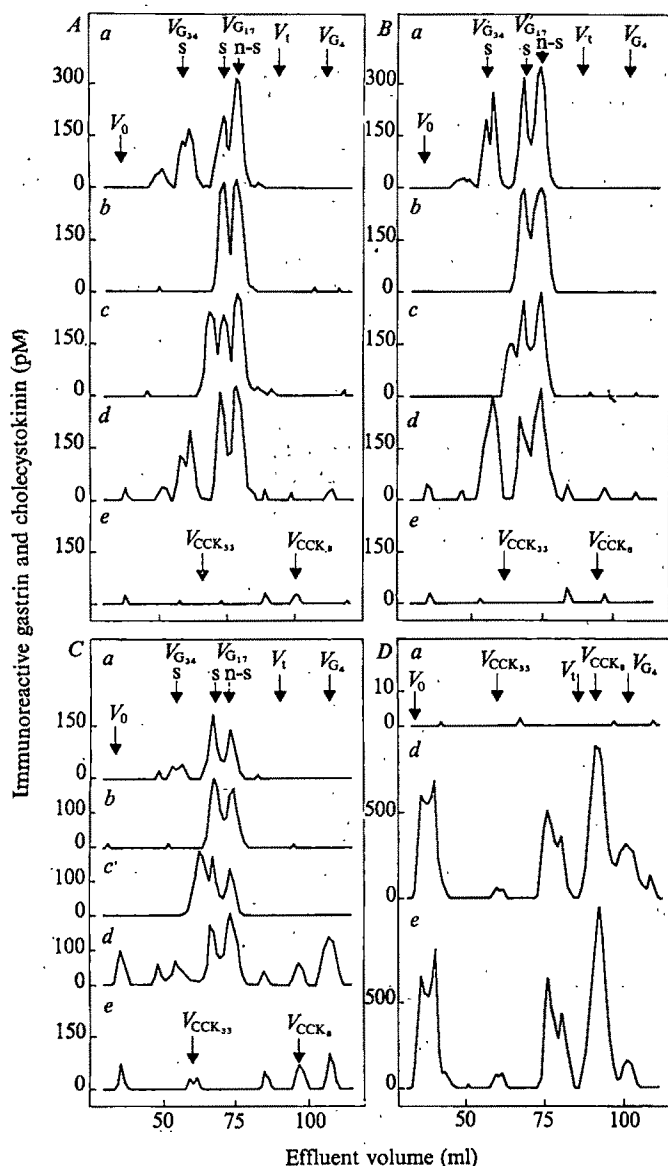
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Localisation of gastrins to neuro- and adenohypophysis

SEVERAL peptides including substance P^{1,2}, somatostatin^{3,4}, the enkephalins^{5,6} and cholecystokinin (CCK)^{7–10} have been found both in nerve cells of the brain and in endocrine cells of the gut. CCK was first demonstrated in brain tissue by a gastrin radioimmunoassay as a peptide different from and smaller than heptadecapeptide gastrin⁷. Using six different gastrin antisera, Dockray provided strong evidence that the reported gastrin immunoreactivity⁷ corresponded to the COOH-terminal octapeptide of CCK⁸, which reacts with certain gastrin antisera because of the identical COOH-terminal pentapeptide sequences of gastrin and CCK. Studies using antisera specific for CCK without cross-reaction with gastrin have now confirmed that CCK is present in large quantities in the brain^{9,10} and that the peptide originally demonstrated by gastrin antisera⁷ indeed corresponded to the COOH-terminal octapeptide of CCK. In contrast, no peptides similar to any of the known molecular forms of gastrin have so far been found in brain tissue. We have used various sequence-specific antisera to distinguish between gastrin and CCK, and we report here that true gastrin also is present in the central nervous system. The only region of the brain containing significant amounts of gastrin was the hypophysis cerebri. Both pituitary lobes contained molecular forms of gastrin closely resembling those found in antrum¹¹.

Extracts were prepared from the central nervous system of pigs. Tissue pieces from different regions, weighing between 0.02 and 2 g, were boiled in water (0.1 g ml⁻¹) at pH 6.6 for 20 min, homogenised and centrifuged (2,000g, 20 min). After decantation of the first supernatant 0.5 M acetic acid was added to the boiled tissue precipitate for further 15 min, followed by homogenisation and centrifugation (2,000g, 20 min). Using specific radioimmunoassays for gastrin and cholecystokinin the concentrations in the mixed supernatants were measured. The only region containing immunoreactive gastrin in significant amounts was the hypophysis (Table 1). Extracts of porcine adenohypophysis, neurohypophysis, of the pituitary stalk and the hypothalamus were applied to calibrated Sephadex G-50 columns monitored by five different sequence-specific gastrin and CCK radioimmunoassays.

Three components of gastrin were found by gel chromatography of extracts from the anterior lobe, posterior lobe and stalk of the pituitary (Fig. 1). The components eluted in positions corresponding to component I, gastrin₃₄ and gastrin₁₇. The gastrin₃₄- and gastrin₁₇-like peaks were biphasic, corresponding to sulphated and nonsulphated hormonal forms¹⁷. The immunochemical identity of the gastrin₁₇-like peak was corroborated by the results obtained with antiserum L-6, specific for intact gastrin₁₇ (ref. 14) (Fig. 1b), and with antiserum 1295 specific for NH₂-terminal sequence of gastrin₁₇ (ref. 15) (Fig. 1c). The pituitary extracts also contained a fragment corresponding to sequence 1–13 of gastrin₁₇ (ref. 15) eluted immediately before gastrin₁₇ (Fig. 1c). CCK was not present in the pituitary lobes, but small concentrations were found in the pituitary stalk (Fig. 1d, e) together with the gastrins. Only very small amounts of gastrin, undetectable by chromatography, were found in hypothalamus (Table 1 and Fig. 1a). In contrast, large amounts of CCK in different molecular forms were present in hypothalamus (Table 1 and Fig. 1). One large molecular form was eluted immediately after the void volume; a small peak eluted as the tritriacontapeptide (CCK₃₃); a larger peak appeared immediately before the salt peak, presumably corresponding to the COOH-terminal dodecapeptide; a major fraction of the immunoreactivity corresponded to the COOH-terminal octapeptide of CCK₃₃; and finally a peak corresponding to the COOH-terminal tetrapeptide of CCK₃₃ appeared with an elution constant (K_{av}) of 1.30. Measurements in extracts of bovine and rat hypophysis revealed true gastrins similar to those described in the pig.



Although proof of structural identity between antral and pituitary gastrin requires sequence analysis of the gastrin-like material from the pituitary gland, several lines of evidence support the contention that the pituitary gastrins are very much alike and probably identical with antral gastrins: (1) Four antisera specific for different sequences of gastrin₁₇ reacted to the same extent with the gastrin-like components in pituitary extracts (Fig. 1) as they do with the antral gastrins^{11,14,15}. (2) By chromatography the elution constants of antral¹¹ and pituitary gastrins were identical (Fig. 1). However, the pituitary gland contained relatively more large molecular forms, component I and gastrin₃₄, than antrum¹¹. (3) Trypsin cleaved the larger molecular forms of gastrin in the pituitary extracts to gastrin₁₇-like material (Fig. 2) in a way similar to that observed for gastrins of antral origin^{18,19}. As previously suggested²⁰, it is likely that the two larger molecular forms, component I and gastrin₃₄, are biosynthetic precursors of the principal gastrin, the heptadecapeptide.

The results presented here add a new hormone to the list of peptides located both in the central nervous system and in the gut. Compared to the other peptides on this list gastrin displays specific features. The regional distribution within the brain is unique in that significant amounts of gastrin were present only in the hypophysis. The other regions of the central nervous system contained no gastrin (Table 1). Brain-gut peptides such as substance P²¹, somatostatin²², neurotensin²³, vasoactive intestinal polypeptide²⁴⁻²⁶ and CCK (Fig. 1 and Table 1) are all present in substantial amounts in the hypo-

Fig. 1 Gel chromatography of immunoreactive gastrins and cholecystokinins in extracts from porcine pituitary and hypothalamus. *A*, Adenohypophysis; *B*, neurohypophysis; *C*, pituitary stalk; *D*, hypothalamus. Frozen pieces of tissue were boiled for 20 min in water (0.1 g tissue ml⁻¹) and homogenised, centrifuged and the supernatant decanted. Equal volumes of 0.5 M acetic acid were added to the tissue which was again homogenised and centrifuged. Aliquots of 1.5 ml of the mixed supernatants from each region were applied to Sephadex G-50 superfine columns (10 × 1,000 mm) eluted at 4 °C with 0.02 M barbital buffer, pH 8.4, containing 0.1% bovine serum albumin at a flow rate of 4 ml h⁻¹. Fractions of 1.2 ml were collected. The columns were calibrated with ¹²⁵I-albumin (void volume, *V*₀) pure sulphated porcine gastrin₃₄ (*V*_{G34-s}), pure sulphated and non-sulphated porcine gastrin₁₇ (*V*_{G17-s} and *V*_{G17-n-s}), pure porcine cholecystokinin₃₃ (*V*_{CCK33}), synthetic porcine cholecystokinin₈ (*V*_{CCK8}), synthetic COOH-terminal tetrapeptide of gastrin and cholecystokinin (*V*_{G4}), and ²²NaCl (total volume, *V*_t). Each sample applied to the column was mixed with trace amounts of ¹²⁵I-albumin and ²²NaCl for internal standardisation. The concentration of immunoreactivity in each fraction was measured by five different radioimmunoassays: *a*, A gastrin assay using antiserum 2604-8, which reacts with the COOH-terminal half of gastrin₁₇ and shows poor reactivity with CCK (<0.002, ref. 12). Antiserum 2604-8 measures component I, gastrin₃₄ and gastrin₁₇ with equimolar potency¹³. *b*, A gastrin assay using antiserum L-6, specific for intact gastrin₁₇ (ref. 14). L-6 is thus not capable of binding either component I, gastrin₃₄ or CCK. *c*, A gastrin assay using antiserum 1295, specific for the NH₂-terminal sequence of gastrin₁₇ (ref. 15). 1295 is thus not capable of binding either component I, gastrin₃₄ or CCK, but binds only gastrin₁₇ and the naturally occurring NH₂-terminal tridecapeptide fragment of gastrin₁₇. *d*, An assay using antiserum 2609, specific for the COOH-terminal tetrapeptide common to both gastrins and CCKs¹². Antiserum 2609 consequently binds both gastrins and CCKs. Monoiodinated ¹²⁵I-synthetic human gastrin₁₇ was used as tracer¹⁰ and pure porcine nonsulphated gastrin₁₇ was used as standard (except in the hypothalamic extract, where CCK₃₃ was used as standard with antiserum 2609). *e*, A CCK assay using antiserum 4698, specific for sequence 25-30 of CCK₃₃. Thus 4698 binds no gastrins, but reacts with CCK₈ and all larger molecular forms of CCK. ¹²⁵I-CCK₃₃ was used as tracer and pure porcine CCK₃₃ was used as standard³³.

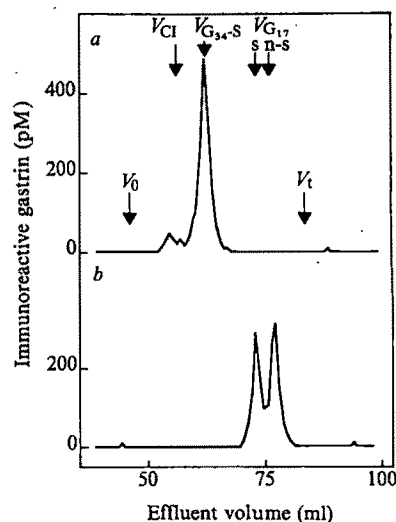


Fig. 2 Gel chromatography of immunoreactive gastrin in extracts from porcine adenohypophysis. Extraction procedure, column chromatography and assay as for Fig. 1. *a*, Elution of an extract containing (atypically) only component I (*V*_{C1}) and gastrin₃₄ (*V*_{G34-s}), but not detectable amounts of gastrin₁₇ (*V*_{G17-s}, *V*_{G17-n-s}) as measured with gastrin antiserum 2604. *b*, Elution of another sample from the same extract after incubation for 20 min at 20 °C with trypsin (Worthington trypsin TPCK, 10 μg ml⁻¹). Trypsin cleavage was terminated by boiling for 10 min, after which the sample was applied to the same column as used for the nontrypsinised sample. The fractions were assayed using gastrin antiserum 2604.

thalamus and other regions of the brain. The differential distribution of gastrin is of particular interest in relation to that of CCK. CCK is present in most brain regions except the epithalamus, cerebellum, and pituitary (Table 1), whereas gastrin is present only in a region free of CCK. We have previously given evidence of a common evolutionary origin of gastrin and CCK in both endocrine cells and neurones²⁷. How and why the common ancestor in antrum and hypophysis develops exclusively to gastrin but in jejunum and the remaining brain to CCK is an intriguing question characteristic for the APUD concept²⁸. The dual localisation of gastrin to both anterior and posterior pituitary lobe is another unusual property, apparently also shared by neurotensin²³. This observation suggests that gastrin is either confined to both endocrine cells (in the adenohypophysis) and nerve terminals

(in the neurohypophysis), or is located in the pars intermedia. Prolactin has also been located to endocrine cells in the adenohypophysis and to nerve terminals, but the nerve terminals were found in hypothalamus²⁹.

The amounts of gastrin in nervous tissue, the pituitary and vagal nerves³⁰, are far below the amounts of gastrin present in antral and duodenal mucosa¹¹. Circulating gastrin thus originates almost exclusively from the antrum and duodenum, and serum gastrin concentrations are reduced more than 10-fold by antro-duodenectomy^{31,32}. The functions of pituitary and vagal gastrin are therefore more likely to be related to local neurotransmitter activity than to more remote effects of the hormones.

I thank Bodil Basse and Gitte Holløse for technical assistance. Antisera were gifts from Drs J. H. Walsh and G. J. Dockray. Peptides for calibrations were gifts from Professor R. A. Gregory and Dr H. J. Tracy, Professor V. Mutt and Dr J. S. Morley. This work was supported by the Danish MRC.

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Table 1 Immunoreactive gastrin and cholecystokinin in regions of the central nervous system of pigs

Region	Gastrin ₁₋₇ Ab. L-6*	Cholecystokinin Ab. 4698†
Telencephalon		
Pallium:		
Frontal lobe	<0.1	556.8±103.0
Parietal lobe	<0.1	1159.0±184.7
Temporal lobe	<0.1	1749.6±209.5
Occipital lobe	<0.1	660.3±78.1
Rhinencephalon:		
Olfactory bulb	<0.1	271.4±48.6
Olfactory area	<0.1	766.3±110.2
Hippocampus	<0.1	136.9±26.3
Corpus striatum:		
Caudate nucleus	<0.1	375.3±53.4
Lentiform nucleus	<0.1	234.0±38.8
Diencephalon		
Thalamus:		
Anterior thalamus	<0.1	24.5±4.6
Posterior thalamus	<0.1	31.0±6.9
Geniculate bodies	<0.1	11.4±0.9
Epithalamus:		
Pineal body	<0.1	<0.1
Thalamic Habenulae	<0.1	<0.1
Hypothalamus:		
Mammillary bodies	<0.1	36.2±10.7
Pituitary stalk*	10.7±0.6	18.4±7.0
Posterior pituitary*	31.6±3.1	<0.1
Anterior pituitary*	19.2±1.5	<0.1
Hypothalamus (remaining part)	0.2±0.1	110.0±26.1
Mesencephalon		
Superior colliculus	<0.1	15.7±3.9
Inferior colliculus	<0.1	52.3±10.2
Nigral substance	<0.1	46.5±11.7
Metencephalon		
Cerebellum	<0.1	<0.1
Pons	<0.1	2.4±0.9
Myelencephalon		
Medulla oblongata	<0.1	15.0±2.7
Spinal cord		
Cervical ventral horn	<0.1	<0.1
Cervical dorsal horn	<0.1	<0.1

Hormone concentrations were determined by specific radioimmunoassay and are expressed as pmol per g wet weight. Values are mean ± s.e.m. for four experiments.

*Antiserum L-6 is specific for intact heptadecapeptide gastrin (ref. 14).

†Antiserum 4698 is specific for amino acid sequence 25–30 of CCK₃₃. The data on 4698 measurements are brought as reference for the specific gastrin data. They include determinations on three pig brains reported elsewhere³³.

‡n = 14.

Received 11 October; accepted 22 December 1977.

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Dual action of erbium on transmitter release at the frog neuromuscular synapse

LANTHANUM-D¹⁻³ and praseodymium⁴ produce very rapid and large increases in miniature endplate potentials (m.e.p.ps) but they also abolish the induction of transmitter release by nerve impulses—endplate potentials (e.p.ps). We show here that erbium (Er³⁺) also a lanthanide has a similar dual action.

The frog sartorius nerve-muscle preparation was used for this study. Intracellular electrodes (3 M KCl, 10–20 MΩ resistance) were inserted into the superficial fibres of the muscle at room temperature. The preparations were perfused continuously by a Ringer solution of the following composition: 112 mM NaCl, 0.4 mM CaCl₂, 5 mM MgCl₂, 24 mM Na CO₃OH, pH 7.2–7.4, which blocks transmission and prevents contraction.

Addition of 5×10^{-4} to 10^{-3} M Er^{3+} (ErCl_3 dissolved in the same Ringer and buffered with HEPES, $\text{pH} = 7$) caused an immediate increase of the m.e.p.p., to a frequency too high to be measured, in the first 2 or 3 min (Fig. 1) and an abolition of e.p.p., without any modification of the postsynaptic potential. These effects were not reversed after washing for 45–60 min in normal Ringer. Er^{3+} at between 5×10^{-4} and 5×10^{-5} M usually caused (depending on the fibre) a rapid increase in m.e.p.p. frequency; after a further 10 to 15 min the m.e.p.p. frequency fell to a level lower than the control (Fig. 2) and remained at that level during a 45 min washing out. At the same time there was an abolition of e.p.p. with a recovery after 20–30 min washing out. These effects remained essentially the same when tetrodotoxin (2×10^{-7} g l^{-1}) was added to the Ringer or, in Ca^{2+} -free Ringer. For the very low Er^{3+} concentration of 15×10^{-6} M the response was a rapid diminution of the quantum content of e.p.p. which remained at this low value, while m.e.p.p. frequency showed no modification or diminished slightly only when the quantum content was at its lowest values (Fig. 3). It should be noted that we never observed the increase of m.e.p.p. frequency associated with an initial

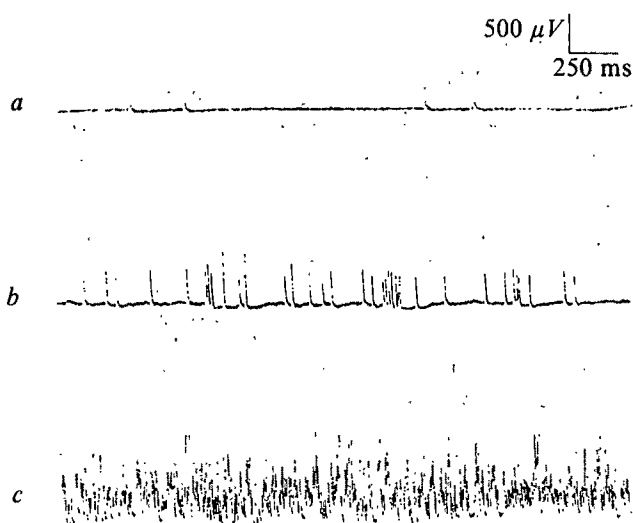


Fig. 1 Effect of 6×10^{-4} M Er^{3+} on m.e.p.p. frequency: *a*, before Er^{3+} ; *b*, after 1 min in 6×10^{-4} M Er^{3+} ; *c*, after 3 min 30. The frequency rises immediately but the maximum frequency is delayed. With 10^{-3} M Er^{3+} this effect is instantaneous.

decrease followed by an increase of quantum content described by Alnaes and Rahamimoff⁴ with the same concentration of praseodymium (but these authors worked with 10–12 mM Mg^{2+}).

The mechanisms of this concentration-dependent action of Er^{3+} are not clear. As Heuser and Miledi² and Miledi⁵ pointed out for lanthane, it seems possible that lanthanides block evoked transmitter release because, by an unknown mechanism, they prevent the inward flux of Ca^{2+} (as the propagated action potentials in synaptic nerve terminals are not blocked², it is not possible to infer a depolarisation block which could have been responsible of both the increase in m.e.p.p. frequency and the abolition of e.p.p.). This effect has been noted in various preparations, and is most marked in frog sartorius⁶. Er^{3+} action on m.e.p.p. and e.p.p. at very low concentrations can be very well explained by this hypothesis. But how does one account for the increase in m.e.p.p. frequency for greater concentrations if no Ca^{2+} can enter the membrane? It has been supposed⁴ that an influx of lanthanides in the nerve terminal would block Ca^{2+} sequestration by the mitochondria, increasing the $[\text{Ca}^{2+}]$ in the cytoplasm of the terminal and enhancing spontaneous release. Although it is well known that Er^{3+} acts on isolated mitochondria at very low concentrations⁷ it seems that this instantaneous action of lanthanides on the whole cell is too fast

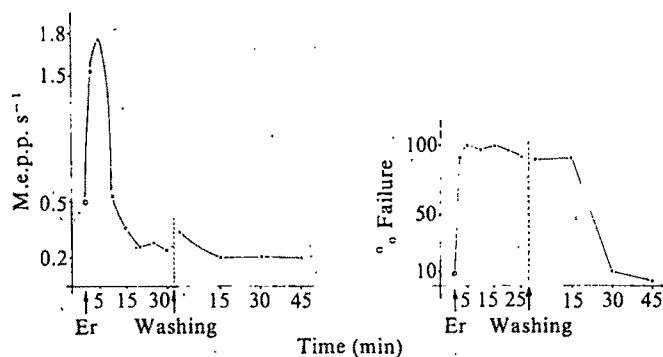


Fig. 2 Effect of Er^{3+} 2×10^{-4} M *a*, Very rapid increase of m.e.p.p. with progressive decline which remains during washing. *b*, Very rapid abolition of e.p.p., but they reappear after 15 min washing in normal Ringer whereas m.e.p.p. frequency is still diminished.

to be explained by this mechanism. Moreover, it is generally accepted that lanthanides remain exclusively extracellular^{8,9}, concentrating in the synaptic folds^{2,10}. However, it has been claimed that there is a slow uptake of rare-earth ions into the axoplasm¹¹, although, here again, this uptake takes hours and nothing is observed in the first few seconds. For this last reason the possibility of an internal screening effect which, following the hypothesis of thermal collision between the membrane and the vesicles, has been supposed to be responsible for enhancement of spontaneous release by multivalent cations¹², seems also, at least for lanthanides, untenable.

A further hypothesis could be supported by the action of the lanthanides on the Na^+ channel; the lanthanides increase the nerve spike duration at Ranvier's node¹³ and crayfish giant axon¹⁴—probably by a sustained activation of the sodium conductance. As it is known that an increase in intracellular $[\text{Na}^+]$ increases Ca^{2+} influx this could be responsible for the facilitation of m.e.p.p. frequency. There are three reasons against this interpretation. First, uranyl, which also prolonged the nerve action potential, enhanced both spontaneous and evoked potentials¹⁵. Second, to test the action of the Na channel, we showed that there is no modification of the enhancement of m.e.p.p. frequency by Er^{3+} in the preparation blocked by tetrodotoxin. So it seems that an increase Ca^{2+} influx by the Na channel is not responsible for the action on spontaneous release. Interestingly, tetrodotoxin does not alter modification of

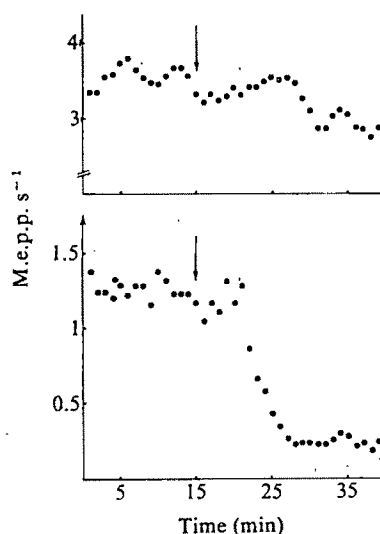


Fig. 3 After the introduction (arrow) of Er^{3+} 15×10^{-6} M, the m.e.p.p. frequency diminishes slightly only when the quantum content (*m*) is very low. Stimulation frequency 0.5 H.z. M.e.p.p. frequency and *m* are calculated with a moving bin of 1 min.

spontaneous release by presynaptic polarisation¹⁶. Third, we found that the action of Er^{3+} still persists in Ca^{2+} -free Ringer.

We suggest that the action of lanthanides on transmitter release is best explained by the hypothesis of a dual site of action of Ca^{2+} on the terminal axon. A phasic action is specific for the phasic release normally induced by nerve impulses¹⁷; this could be the "late Ca channel" of Baker *et al.*, which is inhibited by lanthane¹⁸. Second, a tonic action is responsible for the basic spontaneous rate of release. The tonic action is not strictly Ca -dependent¹⁷. Lanthanides, then, (like other divalent cations) could have a non-specific action on spontaneous release, by means of an external screening effect. This would modify the transmembrane electric field, as d'Arrigo pointed out for crayfish axon¹⁹, and possibly enhance the spontaneous data of release, without any ion influx. The maintenance of a slow rate of spontaneous release, after some minutes and after washing, could be then caused by a new value of the transmembrane potential caused by some ionic rearrangement connect to the nonspecific but tightly bound Er^{3+} . The reappearance of e.p.p. could be explained if the Er^{3+} responsible for the competition with the specific Ca^{2+} site could be more easily washed out.

The finding that a very low concentration of Er^{3+} can block the specific sites of evoked release without acting on spontaneous-release seems a very strong argument in favour of our hypothesis.

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Received 30 June; accepted 19 December 1977.

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Monolayer coupling in sphingomyelin bilayer systems

THE transmission of information across biological membranes is of obvious importance. In the past, discussions of possible mechanisms for transmembrane linkage, in the absence of transport or permeation, have centred on the role of the integral membrane proteins, which function as receptors¹. When the lipids have been discussed in this context, the speculations have centred on their influence on the proteins, by way of a 'viscotropic' effect², induced by changes in ionic strength³ or temperature; that is, it has generally been implicitly assumed that the two lipid monolayers act independently of each other⁴. A detailed nuclear magnetic resonance (NMR) study of the thermal behaviour of small single-walled vesicles composed of synthetic phosphatidylcholines has shown that this is indeed the case for these systems⁵. In this report, we present evidence

that vesicles composed of another class of phospholipid, sphingomyelin, do exhibit coupling between the two monolayers, and thus could be involved in transmembrane communication in biological systems.

Phosphatidylcholine-sphingomyelin molecular differences and their relationship to differences in various physical properties have recently been discussed in some detail^{6–10}. The important difference for this study is that the two methylene chains of sphingomyelin molecules are unequal in length. One chain is part of the sphingosine base, and thus has a fixed length which extends about 13 carbons into the bilayer from the interface region^{6,9}. The other is a fatty acid acylated to the amine of the sphingosine. The fatty acid composition varies according to the source, but 16, 18 and 24 carbon fatty acids are the predominate species^{11,12}. There is therefore a chain length difference of about 3, 5 or 11 carbons, respectively. This is in contrast to the situation for natural glycerophospholipids, which, when the fatty acid composition^{11,12} and the chain shortening effect of the *cis* double bonds are taken into account, are not expected to have markedly asymmetric chain lengths. The limited amount of work done on chain pairing in phosphatidylcholines has shown a rough correlation between the chain lengths of the fatty acids of a given molecule^{13,14}. The chain length asymmetry of sphingomyelin molecules has been implicated as being a possible factor in the usual calorimetric behaviour of natural and mixtures of synthetic sphingomyelins, which do not follow the simple, ideal phase rules applicable to phosphatidylcholine mixtures. For example, the transition temperature of *N*-lignoceryl (C24:O)-sphingomyelin is found to be lower than that of *N*-stearoyl (C18:O)-sphingomyelin⁷. Another consequence of the chain length asymmetry is that the longer acyl chain could extend beyond the midpoint of the bilayer, interdigitating into the other monolayer, which would provide a mechanism for the coupling of the monolayers. The evidence given below indicates that this coupling does occur for *N*-lignoceryl (C24:O)-sphingomyelin.

The experimental approach employed by Sillerud and Barnett⁵ to study monolayer coupling of phosphatidylcholines utilised two properties of trivalent, paramagnetic lanthanide ions: the well-known fact that they can be used to separate the resonances due to molecules on the outside of small single-walled vesicles from those of molecules on the inside¹⁵, and that the binding of these ions to the phosphatidylcholine head groups increases the thermotropic transition temperature¹⁶. Sillerud and Barnett monitored this transition using several different parameters under various conditions, and found that the transition temperature of the outside monolayer can be shifted without changing the temperature of the inside monolayer transition⁵. For this preliminary communication, we have reported only the choline methyl proton line widths, since these resonances give good outside/inside resolution, are of high and constant¹⁷ intensity through the phase transition, and show a reasonably sharp break at a temperature (hereafter referred to as the onset temperature) which corresponds well to the onset temperature of the thermotropic transition of small single-walled vesicles, as monitored by calorimetry¹⁸. We have found, as was to be expected, that the ions do not need to be paramagnetic in order to increase the onset temperature, as La^{3+} , which is diamagnetic, also produces the same effect. In our experiments, we have used mixtures of La^{3+} and Pr^{3+} , as concentrations of paramagnetic Pr^{3+} which give outside monolayer onset temperature increases of 5 °C or more also produce excessive line broadening, so that accurate line width measurements become more difficult.

In their proton NMR study on dimyristoylphosphatidylcholine vesicles, Sillerud and Barnett used Pr^{3+} to phosphatidylcholine ratios of up to 0.3, for which the outside monolayer onset temperature increase is 2.2 °C (ref. 5). We have repeated their experiment using dipalmitoylphosphatidylcholine vesicles and a larger total La^{3+} phosphatidylcholine ratio of 2.4 (the $\text{La}^{3+}/\text{Pr}^{3+}$ ratio was 1.7). The outside monolayer transition temperature was increased by 7 °C. Within the accuracy of the

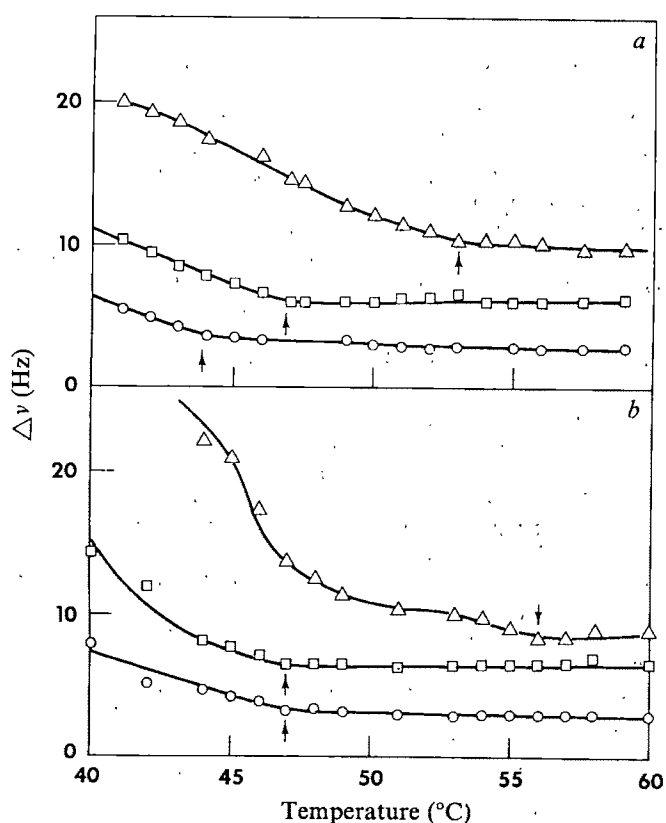


Fig. 1 Temperature dependence of the choline methyl proton line widths of small single-walled vesicles composed of (a) *N*-lignoceryl (C24:0)-sphingomyelin and (b) *N*-stearoyl (C18:0)-sphingomyelin. ○, Outside (downfield) resonance line width in the absence of trivalent ions; □, line width of the resonance due to molecules on the inside of the vesicles in the presence of lanthanide ions; Δ, outside resonance line width in the presence of La^{3+} . The $\text{La}^{3+}/\text{Pr}^{3+}$ ratio was 1.9. The total La^{3+} sphingomyelin ratios were a, 2.2; b, 2.7. Sphingomyelin concentrations were: a, 38.4 mM; b, 29.3 mM; as determined by phosphate analysis. The vesicles were prepared by sonication above the transition temperature in 2-min bursts to optical clarity in 50 mM KCl in D_2O . The samples were centrifuged only briefly to remove titanium particles, and were therefore not homogeneous. Fourier transform NMR spectra were measured at 100 MHz using a JEOL PS-100 P/EC-100 spectrometer. A $180^\circ\text{-}\tau\text{-}90^\circ$ pulse sequence was used to reduce the intensity of the residual HDO resonance²⁴. Conditions used were: 40 scans, 1,000 Hz sweep width, 8K data points, 4.1-s acquisition time, 30- μs 90° pulse, delay between the pulse and the start of acquisition 1,050 μs . The temperature was set using the JEOL VT/3B temperature controller: The temperature was measured before and after each set (2) of spectra with a Yellow Springs Instrument thermistor No. 44016 set in an NMR tube. The precision of the temperature measurements is estimated to be $\pm 0.2^\circ\text{C}$ and the accuracy $\pm 0.5^\circ\text{C}$. The temperatures taken as representing the onset of the thermotropic transition are indicated with arrows. The error limits for the line width measurements are: without La^{3+} , $\pm 0.2\text{Hz}$ above and $\pm 0.4\text{Hz}$ below the onset temperature; with La^{3+} , $\pm 0.4\text{Hz}$ above and $\pm 0.8\text{Hz}$ below the onset temperature.

temperature measurements (0.5°C), there was no change in the onset temperature of the inside monolayer from that observed for both monolayers in the absence of trivalent ions (39°C).

Figure 1a presents evidence that the inside monolayer onset temperature is affected by increasing the outside monolayer onset temperature for *N*-lignocerylsphingomyelin. When the outside onset temperature was increased by 9°C , the inside onset temperature increased by 3°C relative to the onset temperature in the absence of trivalent ions. This partial coupling of the two monolayer transitions remained roughly the same as the trivalent ion/phospholipid ratio was varied. For example, when the outside monolayer onset temperature was increased by 5°C , the inside onset temperature increased by

1°C . The evidence that this effect is due to chain length asymmetry is shown in Fig. 1b, in which data for *N*-stearoylsphingomyelin are plotted. Up to 9°C , increases in the outside monolayer onset temperature produced no measurable effect on the inside onset temperature. In experiments to date, the detailed shape of the outside monolayer line width curve below the onset temperature was not entirely reproducible. We therefore attach no significance to the differences seen between the outside monolayer line width curves for *N*-stearoyl and *N*-lignocerylsphingomyelin. The onset temperatures themselves are, however, completely reproducible.

For these studies, it is important to note that no appreciable fusion or leakiness occurred during the course of the descending temperature runs. This was deduced by re-running the samples at the highest temperature. The line widths, chemical shifts and the integrated intensities of the choline methyl proton resonances (inside/outside ratio), and the fatty acid methylene proton resonances, were within experimental error of the values determined for the initial high temperature spectrum. It should be emphasised that the La^{3+} /phospholipid ratios used in these experiments were much higher than those normally used to produce measurable chemical shifts. For example, a Pr^{3+} /sphingomyelin ratio of 0.07 produces a choline methyl proton shift of 0.6 p.p.m. in beef brain sphingomyelin vesicles⁸. Thus, whereas the control experiments do not categorically exclude the presence of small amounts of La^{3+} inside the vesicles, or alternately, a small proportion of leaky vesicles, the controls would certainly be sensitive to ion concentrations high enough to cause temperature changes.

To suggest possible biological relevance for monolayer coupling, it is necessary to look beyond the systems studied here, as trivalent ions are not found in significant concentrations in biological systems, and as the effect of divalent ions (for example, Ca^{2+}) on zwitterionic phospholipids is difficult to detect¹⁹. However, Ca^{2+} is known to affect markedly the phase transition of charged synthetic phospholipids³, and, more importantly, the fluidity of bilayer systems containing mixtures of neutral and charged natural phospholipids²⁰; and two of the three classes of glycosphingolipids, the gangliosides and the sulphatides, are charged. The glycosphingolipids as a group are components of all mammalian plasma membranes²¹, and they are thought to exist exclusively in the outer monolayer²². Moreover, they have been implicated as being involved in processes such as cell-cell recognition, cell division and transformation, and toxin binding²⁰⁻²³, which can affect membrane fluidity in a manner analogous to the way ions affect it, and, given monolayer coupling, could bring about the transmission of this information to the cell interior.

This work was supported by USPHS grants GM-14628, GM-17452 and HL-17576. We thank Drs Sillerud and Barnett for a pre-print of their work.

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Received 26 September, 1977; accepted 3 January 1978.

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ATP levels modify the activation of the Na pump by external cations in squid axons

It has been proposed that, during the cycle of ATP hydrolysis, the (Na+K)ATPase goes through a 'closed' or 'occluded' conformation¹. This would be produced on the active site for K translocation following dephosphorylation, and, while it lasts, the dephosphorylating cation would remain attached to the enzyme, preventing rephosphorylation. The stability of this conformation varies with the dephosphorylating cations (the more stable complex follows the sequence $\text{Ti}^+ > \text{Rb}^+ > \text{K}^+ > \text{Cs}^+ > \text{NH}_4^+ > \text{Li}^+$). In addition, the complexes are less stable with increase in the ATP concentration¹. Flux experiments in human red cells²⁻⁴ and squid axons^{5,6} have produced some evidence in line with this. Unfortunately, in these cases one cannot be sure if the observed effects were solely the consequence of changes in ATP or were influenced by simultaneous modifications of the ADP and inorganic phosphate content of the cells. We have avoided such difficulties here by using squid dialysed axons, and have found an increased effectiveness of NH_4^+ over K and Rb, and of K over Rb, in activating the Na pump when internal ATP is reduced. This followed the same pattern described for their effects on the levels of phosphoenzyme. Our results suggest the existence of an ATP-sensitive occluded conformation of the Na pump formed after dephosphorylation.

We considered that if the concept of the closed conformation is correct, then the lower the ATP concentration in the cell, the more difficult it should become for a cation with a stable complex with the enzyme to activate the pump from the external side. As the Ti solubility in chloride media is extremely low, and the use of Li would require a large reduction in external Na, the chosen activating cations were K^+ , Rb^+ and NH_4^+ . The washout of internal ATP and other nucleotides was accomplished with excellent results by using glass dialysis capillaries without any metabolic inhibitor. In addition, the ADP resulting from the hydrolysis of exogenous ATP was buffered by adding 5 mM phosphoarginine to the dialysate. As the Na-Na exchange requires the presence of both ATP and $\text{ADP}^{2,6}$, this flux was expected to be minimal with the phosphoarginine buffering system.

Some components of Na efflux other than the Na-K pump would vary at low and high ATP. The Na-Ca and Na-Mg exchange are negligible at low ATP, but considerable at high ATP (ref. 8 and L. J. Mullins and F. J. Brinley, unpublished data). The Na-Ca exchange is abolished in the absence of internal Ca (R. DiPolo, unpublished data). Therefore, all dialysis solutions were Ca-free and contained 1 mM EGTA. The Na-Mg exchange would not be abolished at high ATP and its presence will have to be considered when comparing K-free effects in axons containing low and normal ATP (this would tend to reduce the K-free effect in high ATP axons).

Figure 1 describes a typical experiment. After the washout

period, and on readdition of ATP at 50 μM concentration, the total Na efflux rose to about 7 $\text{pmol cm}^{-2} \text{ s}^{-1}$. Under these conditions, and at a concentration of 10 mM, NH_4^+ was twice as effective as K in promoting an Na efflux above the K-free levels, while Rb did not produce any detectable activation. The rise in the ATP to 3 mM in the dialysate produced an increase in the total Na efflux to about 26 $\text{pmol cm}^{-2} \text{ s}^{-1}$ in 10 K artificial seawater (ASW). A new test on the sensitivity of Na efflux to the external K and K-like cations showed a completely different response: K was at least as effective as NH_4^+ , while at the same time Rb produced a distinct activation over the K-free levels amounting to 50% of that produced by equal concentrations of K. This differential effect of the external cations on Na efflux at low and high ATP is also well illustrated in Table 1, which summarises experiments on three axons. With 50 μM ATP, NH_4^+ is on average 2.6-fold more powerful than K and more than 6-fold more powerful than Rb. On the other hand, with ATP at 3 mM concentration, K becomes at least as good an activator as NH_4^+ , whereas activation due to Rb reaches as much as 70% of that due to NH_4^+ .

The extent to which these effects are due to changes in the maximal rate of Na efflux and to alterations in affinities of the external site toward the activating cations remains unclear. Other experiments (not shown) indicated that the reduction in ATP levels indeed modified those apparent affinities, with the $K_{1/2}$ for [K]_o decreasing from about 8 mM at 5 mM ATP to about 1 mM at 50 μM ATP. The effects reported here at K, Rb and NH_4^+ concentrations of 10 and 20 mM, therefore seem much more likely to be due to alterations in the maximal rate of pumping than to those affinity changes.

Also noticeable in Fig. 1 is the reduction in the K-free effect (fractional drop in the total Na efflux on removal of external K)

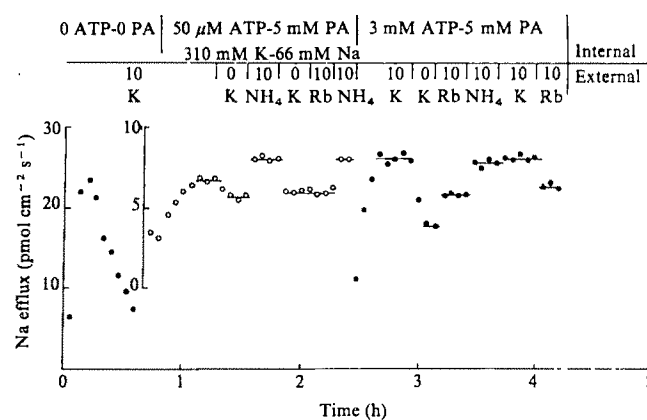


Fig. 1 The effects of internal ATP on the external monovalent cation activation of Na efflux in a dialysed squid axon (axon 270577-A, 475). The composition of the dialysis solution was (mM): Na^+ , 66; K^+ , 310; Mg^{2+} , 4 in excess of the ATP concentration; Tris^+ , 5; Cl^- , 79; aspartate⁻, 310; EGTA⁴⁻, 1; glycine, 330. The osmolarity was 980 mosm and the pH (20 °C) 7.1. ATP and phosphoarginine (PA) were obtained from Boehringer as sodium salts; they were neutralised with Tris (ATP) and HCl (PA) to pH (20 °C) 7.1 and stored as 250 mM solutions at -90 °C. The ATP solution contained in addition 250 mM of MgCl_2 . The Na^+ concentration referred to above is that acquired after the addition of all constituents and was maintained at the same concentration regardless of the amounts of ATP and PA. The composition of the standard artificial seawater was (mM): Na^+ , 440; K^+ , 10; Mg^{2+} , 50; Ca^{2+} , 10; Tris^+ , 10; Cl^- , 580; EDTA⁴⁻, 0.1. The osmolarity was 1,050 mosm and the pH (20 °C) 7.6. The removal or replacement of external K was made without changing the other constituents. At zero time, the dialysis began with the indicated solution plus radioactive Na. The apparent rise in Na efflux between 0 and 12 min does not represent a real flux increase, but the time taken for the isotope to reach steady-state distribution. The reduction which follows is a consequence of the ATP washout. The external solution flow was about 1 ml min^{-1} at a temperature of 17.5 °C. ●, Levels of flux indicated on the left ordinate axis; ○, flux indicated on the inserted axis. More details on the dialysis technique can be found in ref. 7.

Table 1 Stimulating effect of cations on sodium efflux at different ATP concentrations

	50 μ M ATP			3 mM ATP		
	K	Rb	NH ₄	K	Rb	NH ₄
270577-A	0.43	<0.1	1.0	1.06	0.5	1.0
280577-A	0.32	0.15	1.0	1.03	0.75	1.0
280577-B*	—	0.23	1.0	—	0.88	1.0
Mean	0.38	<0.16	1.0	1.05	0.71	1.0
Relative to K	1.0	<0.42	2.63	1.0	0.68	0.95

*The stimulating cation concentration was 20 mM. In all other cases the concentration was 10 mM.

The general experimental procedure, including ATP depletion and repletion, is described in detail in the legend for Fig. 1. The individual values for the cation-activated Na efflux were normalised in relation to those with NH₄⁺ as the activating cation. In the last row, the means normalised in relation to K activation are given.

which followed the reduction in ATP. From the total number of experiments carried out, the average K-free effect decreased from 0.38 ± 0.03 (s.e.m.) ($n = 15$) with 3–5 mM ATP to 0.13 ± 0.04 ($n = 9$) with an internal ATP of 20–50 μ M. In no case was a reversal of the K-free effect seen (larger Na efflux in K-free than in K-containing seawater). Such reversal was reported in conditions which not only reduced the ATP levels, but also must have had large amounts of ADP and inorganic phosphate²⁻⁶. This seems to indicate that the reversal response is a partial relief of inhibition (by removing external K) of a Na-Na exchange which requires ATP and ADP. In the present experiments then, one sees the responses of the pump activation solely as a consequence of changes in ATP.

This brings the present experimental conditions closer to those of *in vitro* ATPase reported by Post¹.

Our results therefore agree with the idea of enzyme-cation complexes with different degrees of stability formed after dephosphorylation. With an apparent K_m for phosphorylation of about 1 μ M ATP⁹, it is obvious that these experiments describe an ATP effect on a regulatory or control site with much lower affinity (see also ref. 1). This control site regulates the stability of the occluded conformation, and through that, the further events of ATP binding, phosphorylation and cation translocation.

This work was supported by grant BNS 76-81050, from the US NSF to L.A.B. We thank the Director of The Marine Biological Laboratory, Woods Hole, for the facilities placed at our disposal.

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Transmission of Creutzfeldt-Jakob disease with scrapie-like syndromes to mice

THE transmission of Creutzfeldt-Jakob disease of man, one of the subacute spongiform virus encephalopathies¹, to guinea pigs^{2,3} and to hamsters⁴ has been reported from this laboratory; Brownell *et al.* have also claimed transmission of Creutzfeldt-Jakob disease to mice^{5,6}. Here we present

evidence that mice are susceptible to this transmissible encephalopathy and that there are similarities between the clinical and pathological findings in experimental Creutzfeldt-Jakob disease in mice, and those observed in the same host in experimental scrapie⁷⁻¹¹.

Eleven 2-month-old Swiss mice (an outbred CD1 strain, obtained from Charles River and bred at Yale) were inoculated with 0.05 ml intracerebrally and 0.1 ml intraperitoneally (i.p.) of a 10-fold diluted normal saline suspension of brain from a guinea pig which developed Creutzfeldt-Jakob disease during the second passage of the disease. Clinical disease appeared in mice between 334 and 631 d after inoculation. From two diseased mice showing subtle clinical signs, and killed at 391 and 464 d, a similar suspension was prepared and the same amount of inoculum as that used in the first passage was again injected into seven mice of the same age and strain intracerebrally and i.p. (second passage). Signs of disease occurred during the second passage between 402 and 549 d after inoculation. No reduction of the incubation period between the first and second passage in mice was observed. A third passage is in progress and 10 weanlings and 12 adult mice of the same strain were again inoculated from material originating from a mouse which developed a scratching syndrome during the second mouse passage.



Fig. 1 Mouse in process of scratching back of neck. Note destruction of skin. This lesion developed in last 2 weeks of disease.

The clinical signs and syndromes that developed in experimental Creutzfeldt-Jakob disease in mice are similar to those described in mice inoculated with scrapie⁷⁻¹¹. Most of the inoculated mice had ruffled coats, arched backs, dullness, reluctance to move, and uncoordinated movements of the hind legs. Occasionally hyperactive animals were noted running in the cages, especially when handled. These clinical signs were subtle, nonspecific and of relatively short duration (2–4 d). All these signs have been described by Chandler^{7,8} and Pattison and Smith¹⁰ in mice inoculated with scrapie.

In the first passage of Creutzfeldt-Jakob disease in mice, two of eleven, and in the second passage, five of seven animals, developed a scratching syndrome. These mice violently and compulsively pawed at and scratched the upper part of the back in the lower cervical and upper thoracic regions. The scratching caused the skin to become excoriated, ulcerated and covered with bloody scabs (Fig. 1). The scratching syndrome lasted up to 2 weeks. The animals also licked, nibbled, and chewed the paws of the forelimbs in stereotype fashion. Scratching at exactly the same region has also been recorded in individual mice inoculated with scrapie¹². Scratching was a prominent feature in the mice inoculated with scrapie by Morris and Gajdusek⁹ and the authors drew a parallel "to the reactions

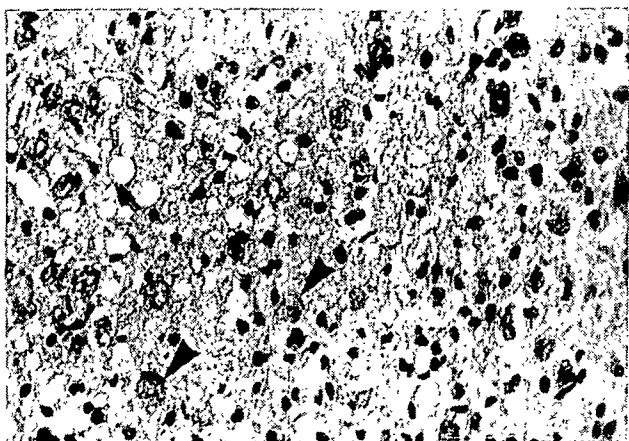


Fig. 2 Cortex. Spongy changes with increased numbers of glial cells (microglia and astrocytes). Only a few neurones remain (examples shown by arrows). Staining by haematoxylin and eosin. $\times 655$.

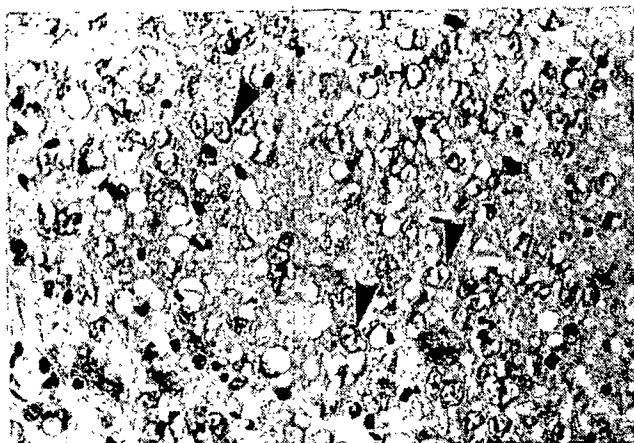
seen in sheep with scrapie¹³ and attributed by Gordon to itching¹³.

Five of the eleven animals of the first passage were found dead, at 334, 369, 449, 557 and 588 d after inoculation. One of these mice developed the scratching syndrome, but others died without showing any detectable clinical signs. The six remaining mice of the first passage were killed at 386, 391, 463, and 631 d, one mouse had the scratching syndrome and the remaining five had only subtle clinical signs. During the second passage, one mouse with the scratching syndrome was found dead, four with the scratching syndrome were killed at 516, 521, 538 and 549 d, and two with only subtle signs were killed 445 and 456 d after inoculation. Changes in body weight were not grossly apparent in the mice infected with the Creutzfeldt-Jakob agent.

Gross and microscopic examination of the skin in affected mice did not show any mites or other parasites. Our mouse colony at Yale is totally free of any ectoparasites. At autopsy, two animals of the first passage and one mouse of the second passage showed a conspicuous and maximal dilation of the urinary bladder. Dickinson recorded the same finding in mice inoculated with some strains of scrapie¹⁴.

No gross findings were detected in the central nervous system. Microscopic lesions, however, were similar to those recorded in mice infected with scrapie¹⁻¹². Neuronal and astrocytic changes and status spongiosus were present in mice inoculated with the Creutzfeldt-Jakob agent. These

Fig. 3 Caudate nucleus. Severe spongy changes (vacuoles), neuronal loss and several pale astrocytes (examples shown by arrows). Staining by haematoxylin and eosin. $\times 610$.



lesions were observed predominantly in the cerebral cortex, basal nuclei and thalamus (Figs 2 and 3). The cerebellum, mesencephalon and pons were mildly affected. Degenerative changes and loss of nerve cells were more pronounced in the convexity than in the base of cerebral cortex. In the cerebral cortex as well as in the basal nuclei, several small vacuoles were seen in the neuropil. No definitive vacuoles in the white matter were observed and no senile (amyloid) plaques were seen. Concomitant with the neuronal loss, there was a moderate increase in numbers of astrocytes. A mild to moderate increase in microglial cells could also be seen in affected regions. No diffuse or perivascular infiltrates composed of lymphocytes or plasma cells could be detected in our slides. In mice of the second passage, the distribution and type of lesions were similar to those described in the first passage.

None of the four control mice inoculated with normal guinea pig brain developed any of the above signs and syndromes and on microscopic examination they showed no central nervous system changes. The possibility that the above clinical signs and the lesions in mice infected with the Creutzfeldt-Jakob agent are due to contamination with scrapie can be excluded, as no investigator at Yale has been or is currently working with scrapie and no members of our laboratory have ever been exposed to this agent.

Now that Creutzfeldt-Jakob disease has been transmitted to mice, it will be possible to make many detailed comparisons with scrapie which would otherwise be impossible. Since there are several strains of the scrapie agent, each with its own variant of the disease, it is conceivable that Creutzfeldt-Jakob disease transmitted to guinea pigs, hamsters and mice may display similar variants; some of these Creutzfeldt-Jakob strains may be closer to the scrapie agent than others.

We thank Phyllis Johnson, Margaret von Ehr and Elizabeth Mullaly for assistance. This work was supported by USPHS grant NS-12674.

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α -Crystallin polypeptides as markers of lens cell differentiation

THE acquisition of specific biochemical characteristics during cell differentiation is thought to be due to differential gene activation, and the vertebrate eye lens provides a useful tool for investigation of the process^{1,2}. The lens arises from epithelial cells arranged in a monolayer at the anterior side of the organ and on differentiation they pass through an elongation zone, becoming fibre-like concomitant with a large increase

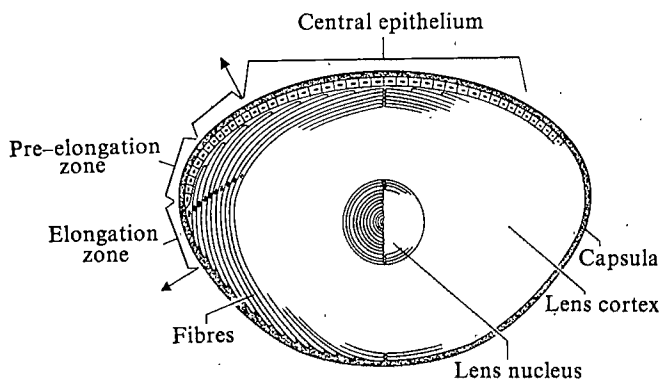


Fig. 1 Schematic drawing of a vertebrate eye lens. Cells in the epithelial layer divide and contribute to the elongating cells that are displaced to the lens cortex.

in volume and rapid synthesis of crystallins (Fig. 1). One of the prominent lens proteins, α -crystallin (composed of subunits αA_1 , αA_2 , αB_1 and αB_2)³ has been suggested as a marker for various biological processes, particularly terminal differentiation, because changes in the subunit composition occur during the transition from epithelial to fibre cells. Delcour and Papaconstantinou reported a change in the stoichiometry of the αB_2 and αA_2 chains of α -crystallin during differentiation from 1:2 in the epithelial cell to 1:3 in the lens fibres⁴. However, changes in subunit composition also occur with ageing of the fibre cell due to the alteration of pre-existing subunits in that

αA_1 and αB_1 probably arise from deamidation of αA_2 and αB_2 ^{5,6}. Furthermore, other chains are formed by post-translational degradation starting from the C-terminus of pre-existing polypeptides^{7, 8}. We have now studied protein synthesis in two different parts of the epithelial monolayer and found further evidence for differential gene activation such that in the central region more αB_2 is synthesised than αA_2 .

Epithelial cells were labelled for 15 h with L-³⁵S-methionine (25 μ Ci, specific activity 180 Ci mol⁻¹) in labelling medium (Hanks' basic salt solution supplemented with 10 % dialysed calf serum and amino acids except for methionine). For each incubation the epithelial cells from ten capsules were used. A maximum of 25 % of the radioactivity added was incorporated. After incubation protein was isolated by freezing and thawing of the epithelial cells which had been transferred previously to water.

Electrophoresis was performed in the first direction on polyacrylamide gel containing 6M urea in 13-cm tubes as described before⁹. The gel was then sliced longitudinally as described before¹⁰. The inner slice was used as the sample gel for the run in the second dimension on a sodium dodecylsulphate polyacrylamide gel according to Laemmli¹¹. This slice was kept in position by pouring a stacking gel solution (5 % acrylamide) on top of the gel slab. Gels were autoradiographed for 72 h. Kodak X-ray RP/R2 film was used.

Hitherto the αA_2 chain has been considered to be quantitatively the most important α -crystallin polypeptide. Our experiments with fibre-free epithelial cells clearly show (Fig. 2) that before the onset of fibre differentiation αB_2 was the predominant α -crystallin subunit. The ratio of αB_2 to αA_2 was as

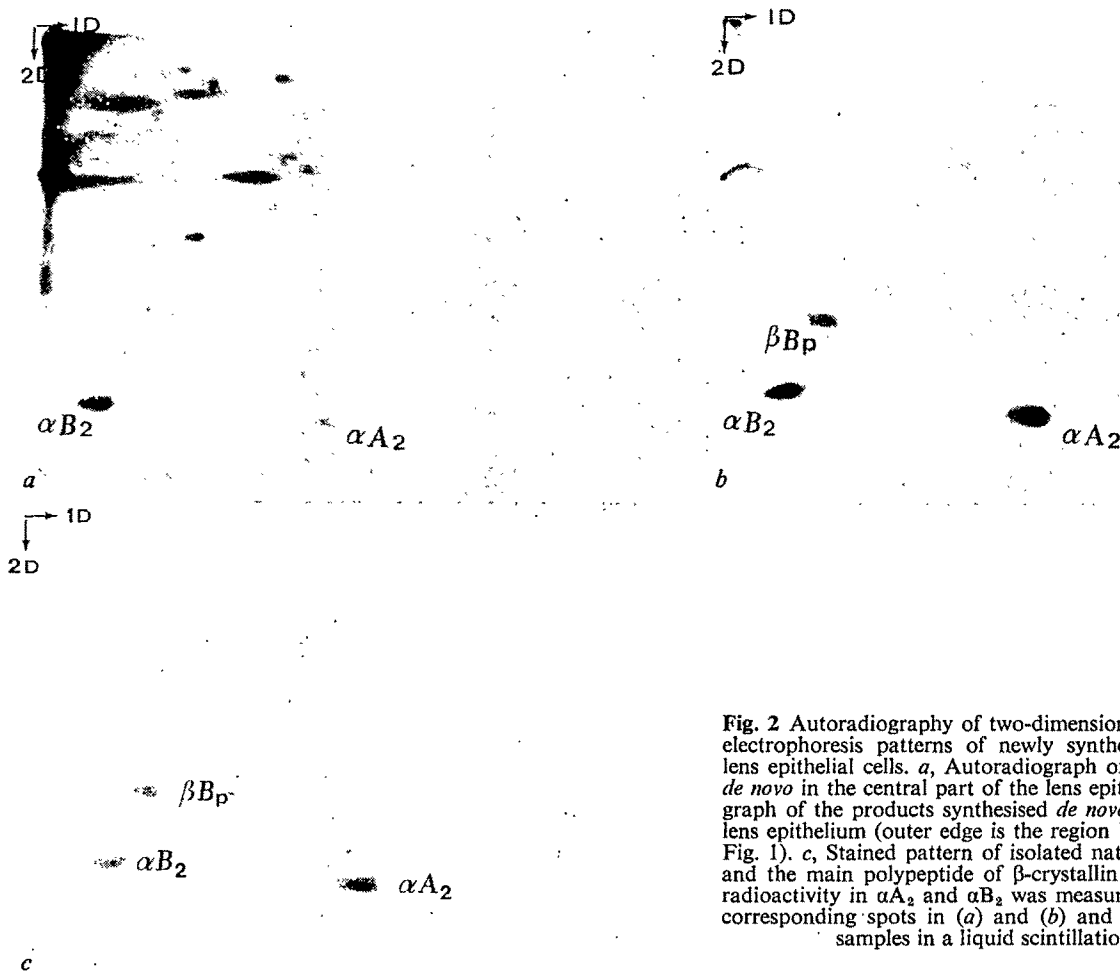


Fig. 2 Autoradiography of two-dimensional polyacrylamide gel electrophoresis patterns of newly synthesised protein in calf lens epithelial cells. *a*, Autoradiograph of products synthesised *de novo* in the central part of the lens epithelium. *b*, Autoradiograph of the products synthesised *de novo* in the outer edge of lens epithelium (outer edge is the region between the arrows in Fig. 1). *c*, Stained pattern of isolated native α -crystallin chains and the main polypeptide of β -crystallin βB_p . The amount of radioactivity in αA_2 and αB_2 was measured by cutting out the corresponding spots in (a) and (b) and counting the isolated samples in a liquid scintillation counter.

Fig. 3 (Left). The products newly synthesised in the reticulocyte cell-free system supplemented with lens fibre polyribosomes (a) compared with the products formed in the central epithelium (b). The heterologous system was incubated as described before¹². Electrophoresis was carried according to Laemmli¹¹.

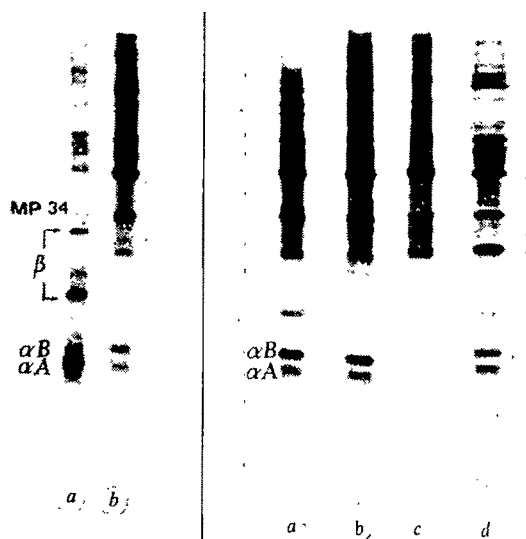
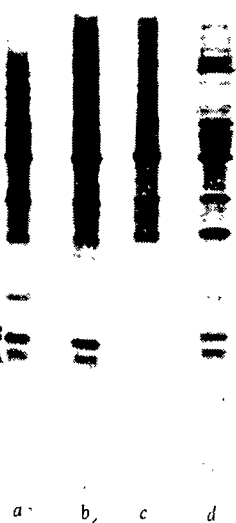


Fig. 4 (Right). Sodium dodecyl sulphate-polyacrylamide gel electrophoresis of newly synthesised lens proteins. a, Non-cultured isolated central epithelium; b, central epithelium cultured on the lens capsule; c, central epithelium cultured on plastic; d, central epithelium cultured on capsules and preincubated with actinomycin D (5 µg ml⁻¹) for 16 h. (The same pattern was obtained with non-cultured cells.)



high as 3:1 in the central epithelium whereas in the outer region of the epithelium a ratio of 2:3 was observed.

To investigate further the mechanisms involved we have compared the pattern of protein formation in lens fibres and epithelial cells. We used lens fibre polyribosomes translated in a reticulocyte lysate because lens fibre cells cannot be maintained in culture. We have already shown that the complete set of lens fibre proteins is synthesised in this cell-free system¹² (Fig. 3). Preincubation of epithelial cells with actinomycin D, in a concentration that inhibits RNA synthesis (5 µg ml⁻¹), left the ratio of newly synthesised α -crystallin chains αA_2 and αB_2 virtually unaffected. The ratio of α -crystallin to non-crystallin proteins also remained unaffected (Fig. 4d). This rules out the possibility that different messenger stabilities are responsible for the observed stoichiometry of the αB_2 and αA_2 chains in the central epithelial cells or for cessation of α -crystallin synthesis in cells grown on plastic¹³. It seems more likely that differential gene activation is involved.

This work was supported in part by the Netherlands Foundation for Chemical Research and the Netherlands Organisation for Pure Research.

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Received 21 June 1977; accepted 11 January 1978.

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DNA polymerases during postnatal myocardial development

THE rate of mitotic division of cardiac muscle cells declines rapidly during late embryonic and early postnatal development¹⁻³ *pari passu* with a progressive restriction in proliferative capacity^{2,3}. Adaptation of adult myocardium to increased functional demand is thus limited to hypertrophy, that is, an increase in size of individual cells without a change in their number. In contrast, non-muscle cardiac cells (interstitial and endothelial cells) retain their capacity to proliferate in response to appropriate stimuli⁴. The mechanisms controlling proliferation in the developing myocardium are not known. Several investigators have noted, however, a close temporal correlation between the age-dependent decline in mitotic activity and reduced levels of enzymes involved in DNA synthesis⁷⁻⁹. The possibility has been raised⁵ that this enzyme loss is, in fact, responsible for the inability of adult myocardial cells to undergo mitosis. Here we describe differential changes in the activities of DNA polymerases from cardiac muscle and non-muscle cells during the postnatal development of the rat heart and suggest that changes in these enzymes are not sufficient explanation for the restriction in proliferative capacity.

Experiments were carried out using male Holtzman rats aged 1-240 d. Myocardial cells were separated from non-muscle cells by a modification of Young's procedure¹⁰; muscle cells make up 93-97% of the final preparation (as judged by microscopy), and 85-90% of these extrude Trypan blue. The proportion of muscle cells does not differ in the age group studied. A progressive increase in the number of both muscle and non-muscle cells was noted with advancing age (Fig. 1). The increase in myocardial cell number reached a plateau near the second week of life, whereas the number of non-muscle cells continued a slower rise till adulthood. The ratio of non-muscle to muscle cells increased from 0.81 at day 1, to 4.2 in adulthood (6 months old).

Three different forms of DNA-dependent DNA polymerases were separated by differential centrifugation (Fig. 2), and on the basis of their sensitivities to *N*-ethylmaleimide, 100 mM NaCl, 50 mM phosphate, and ability to utilise synthetic templates. They correspond to polymerases α , β , and γ , described in other tissues¹¹. Although it has been thought that polymerase α is primarily involved in DNA replication, whereas polymerase β is concerned with DNA repair, assignment of physiological roles to the different forms of the myocardial enzyme is, at present, conjectural.

Figure 1 shows the age-dependent course of myocardial DNA polymerase activity expressed as units per mg protein. Although slight differences exist between the three polymerases, all show a progressive decline with age. The preferential decrease in polymerase α activity reported by Claycomb⁸ was not confirmed either by us or by Huebscher *et al.*⁹.

The results differed significantly when the number of cells in the preparation studied was taken into consideration (Table 1). Although a 35% decline in polymerase activity was noted between days 1 and 240, this is considerably smaller than that previously reported^{8,9} or what might be

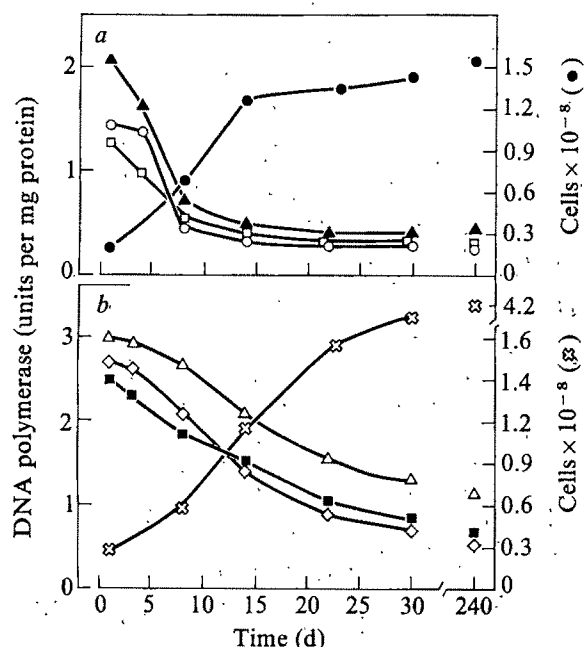


Fig. 1 Time course of DNA polymerase activities of myocardial (a) and non-muscle cells (b). The reactions were carried out for 30 min at 37 °C as described in Fig. 2. Results for polymerase α (▲, △), β (○, ◇) and γ (□, ■) are given for six experiments in each age group; one DNA polymerase unit incorporates 1 nmol ^3H -TTP into acid-insoluble material per hour. The time course of the increase in the numbers of muscle (●) and non-muscle (⊗) cells is also shown.

expected from Fig. 1. The reason for this discrepancy may be that, during this time period, significant changes take place in both the number and size of cardiac cells. Expression of enzymatic activities as units per mg protein would therefore overestimate the decline in DNA polymerase activities with age. In addition, most of the decrease in DNA polymerases occurred during the first 10 d, with a subsequent levelling off. Studies by Neffgen and Korecky¹² on anaemic rats, and by Dowell and McManus¹³ with the aortic constriction model, have clearly shown that at 3–4 weeks of age rat myocardial cells retain a significant capacity for proliferation in response to increased work load; at 6 months of age, this capacity is lost despite no significant interim change in DNA polymerase activity (Table 1). Obviously, factors other than the DNA replicative enzymes must be involved. This is also suggested by the fact that DNA polymerases from non-muscle cells show a significant age-dependent decline (Fig. 1) when expressed as units per mg protein. This is surprising in view of the fact that non-muscle cell number increases during the same period; and that non-muscle cell proliferation in response to stimuli is possible through adulthood^{12,13}. The dilemma is readily resolved by the data in Table 1 which fail to demonstrate a fall in DNA polymerase activity of interstitial cells with age.

Two distinct processes take place during the early stages of postnatal development. One is the decline of mitotic activity as the (predetermined) number of cardiac cells is reached. This probably is a process common to all organs as they reach their normal cell complement and is reflected

in the rapid decrease in ^3H -thymidine incorporation into myocardial cells and, perhaps, the decline in DNA polymerase α activity. The second process is concerned with the loss of the capacity of the myocardium to respond to increased demands with an increase in cell numbers (hyperplasia). The mechanisms involved are unknown but our results indicate that a decline in DNA synthetic activity is not an adequate explanation. In fact, a limited increase in DNA content of myocardial cells does occur with cardiac overload in adult animals¹⁴, even though it does not result in mitosis.

Increasing polyploidism of myocardial nuclei with age¹⁵ or in response to increased functional demands¹⁶ is a reflection of the same phenomenon. The undisputed restriction of mitotic capacity of adult cells may be secondary to changes

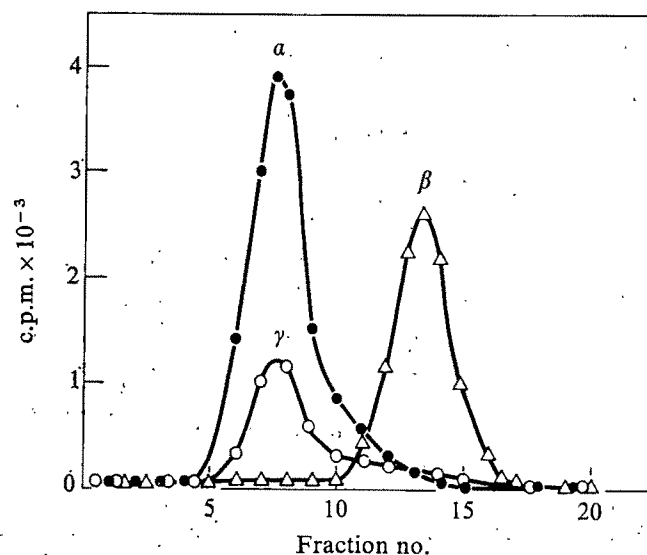


Fig. 2 DNA polymerases from rat left ventricular myocardium. Isolated cardiac muscle cells were separated according to Young¹⁰ and were homogenised in a solution containing 10 mM phosphate buffer (pH 7.5), 10 mM KCl, 1.5 mM MgCl₂ and 0.5 mM dithiothreitol. The homogenate was centrifuged at 100,000g for 1 h at 0 °C and the supernatant was layered on top of a 5–20% sucrose (w/v) gradient in 0.1 M KH₂PO₄ (pH 7.2), 0.1 M KCl and 1 mM dithiothreitol. After centrifuging for 16 h at 100,000g, fractions were collected and assayed for DNA polymerase activity. The assay medium for polymerase α contained 20 mM KH₂PO₄ (pH 7.2), 0.1 mM EDTA, 0.5 mM dithiothreitol, 10 mM MgCl₂, 200 $\mu\text{g ml}^{-1}$ 'activated'¹⁷ calf thymus DNA, 8×10^{-5} M each of dATP, dCTP, dGTP and 1.6×10^{-5} M ^3H -TTP (0.5 Ci mmol⁻¹). The assay for polymerase β was carried out in 50 mM Tris-HCl (pH 8.5), 0.1 M KCl, 10 mM MgCl₂, 1 mM dithiothreitol, 8×10^{-5} M each of dATP, dCTP, dGTP and 1.6×10^{-5} M ^3H -TTP. Finally, the medium for polymerase γ contained 50 mM Tris-HCl (pH 8.5), 50 mM KH₂PO₄ (pH 8.5), 0.13 M KCl, 0.5 mM MnCl₂, 1 mM dithiothreitol, 50 $\mu\text{g ml}^{-1}$ poly (rA):(dT)₂₅₋₃₀ and ^3H -TTP. The reaction was routinely carried out at 37 °C for 30 min and was stopped with 0.5 ml 1 N NaOH, 100 μg heat-denatured calf thymus DNA and 1 mg serum protein. The incorporation of ^3H -TTP into DNA was measured as described by Lynch *et al.*¹⁸. Recovery of the polymerases present in the cardiac homogenate was 72% for polymerase α , 65% for β and 62% for γ ; there was no significant difference in enzyme recoveries between the age groups studied.

Table 1 Age-dependent changes in the activities of DNA polymerases from cardiac muscle and non-muscle cells

Age (d)	DNA polymerase activity (nmol ^3H -TTP/10 ⁶ cells)							
	α		β		γ			
	Muscle	Non-muscle	Muscle	Non-muscle	Muscle	Non-muscle		
4	43.6 \pm 3.1	52.6 \pm 4.0	26.5 \pm 3.8	32.1 \pm 4.2	12.2 \pm 2.3	19.7 \pm 5.2		
14	28.7 \pm 4.6	48.9 \pm 4.2	18.8 \pm 4.4	29.6 \pm 4.1	10.3 \pm 1.9	18.2 \pm 4.8		
28	26.9 \pm 3.2	46.9 \pm 3.7	16.3 \pm 3.3	28.7 \pm 6.0	9.8 \pm 2.2	15.3 \pm 3.7		
240	25.8 \pm 2.6	45.3 \pm 2.1	15.0 \pm 3.1	28.2 \pm 5.5	9.1 \pm 3.1	14.8 \pm 4.4		

Reactions were carried out at 36 °C for 30 min as described in Fig. 2. Results represent mean \pm s.e.m. for six experiments.

in the organisation, composition and function of myocardial chromatin. We have recently studied these parameters during the first few months of age. A progressive decrease in chromatin transcriptive capacity, susceptibility to DNase I digestion, and poly(L)lysine binding occurs in parallel with the restriction in proliferative capacity. Derivative melting profiles of chromatin showed diminished melting of DNA regions bound by non-histone proteins with a concomitant increase in histone-bound regions, as age advanced. These findings are indicative of a decline in DNA accessibility for gene transcription which accompanies and perhaps determines the loss of mitotic potential by the myocardial cells.

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Photochemical (8→8) coupling of purine nucleosides to guanosine

IN comparison with the pyrimidine bases of nucleic acids, the purines are relatively inert to photochemical alteration. No photoproducts derived specifically from adenine or guanine have been isolated from ultraviolet-irradiated DNA or RNA. However, purine bases and nucleosides can be substituted at C8 by free radical species generated photochemically from simple alcohols, amines, and ethers, and these reactions may be relevant to the radiation-induced cross-linking of proteins, and other biological molecules, to nucleic acids¹. We report here that guanosine is substituted in a similar manner by the free radicals produced on photolysis of 8-bromopurine nucleosides to give compounds in which two purine nucleoside moieties are coupled together through their respective C8 positions.

In this way, we have prepared 8-(8-guanosyl)guanosine (GG), 8-(8-guanosyl) adenosine (AG), and 8-(8-guanosyl) inosine (IG); the partial characterisation of AG has been reported previously². These compounds, whose structures are shown in Fig. 1, all exhibit intense fluorescence emission in the region of 410 nm on excitation at wavelengths close to 330 nm. Their formation demonstrates the feasibility of reactions between purinyl-free radicals and purine bases and these may be important in the photochemistry and radiation chemistry of nucleic acids. Furthermore, the simple photochemical coupling reaction described here should be applicable to the synthesis of new fluorescent nucleoside and nucleotide analogues, and to the fluorescent labelling of purine polynucleotides.

Ultraviolet irradiation of equimolar mixtures (both components ~ 0.3 mM) of guanosine with 8-bromoguanosine,

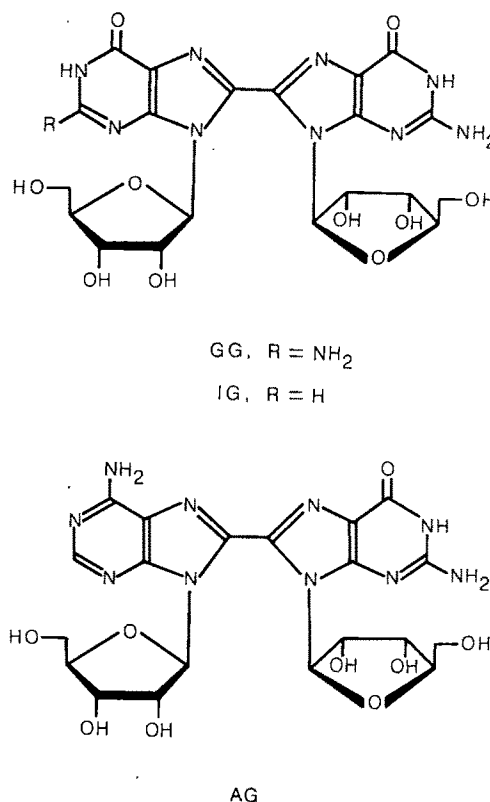


Fig. 1 Structures of the (8→8) coupled purine nucleosides GG, IG, and AG.

8-bromoadenosine and 8-bromoinosine leads to the formation of the (8→8) coupled purine nucleosides GG, AG, and IG respectively. Although the reaction occurs in aqueous solution on irradiation at 254 nm (low pressure mercury lamp), higher yields have been obtained by using 30% aqueous acetone as solvent and irradiating at wavelengths > 290 nm (medium pressure mercury lamp with Pyrex filter) under nitrogen. In each case, the coupled nucleoside photoproduct has been separated from the starting materials by dry-column chromatography³ on silica gel using a solvent mixture of ethyl acetate–water–1-propanol (4 : 3 : 1, upper phase) as eluant. Subsequent purification to chromatographic and electrophoretic homogeneity has been achieved by crystallisation from water for GG, chromatography on Sephadex G-10 for AG, and preparative paper chromatography (Whatman 3 MM, 1-propanol–water, 10 : 3) for IG.

The molecular weights of GG and AG have been established by ²⁵²Cf-plasma desorption mass spectroscopy⁴. Their low field ¹H-NMR spectra (in (CD₃)₂SO containing D₂O) show that both purine bases have been substituted at C8. Thus the spectrum of GG exhibits no aromatic resonances and a doublet at δ 6.21 (J = 6 Hz) for the anomeric protons, while that of AG shows a singlet integrating for 1 proton at δ 8.19, assigned to C2-H of the adenine ring, and two doublets (J = 6 Hz) centred at δ 6.22 and δ 6.38 due to the anomeric protons of the two ribosyl groups. Consistent with their extended conjugation, the ultraviolet absorption of these compounds (Table 1) extends to much longer wavelengths than that of the parent purine nucleosides and is associated with intense fluorescence emission maxima in the range 405–415 nm.

As yet, only very limited quantities of IG have been isolated in pure form. Its structure has been assigned on the basis of its mode of preparation, and the similarity of its chromatographic behaviour and spectroscopic properties (Table 1) to those of GG and AG. Furthermore, on treatment with nitrous acid, AG is partially converted to a product chromatographically identical to IG.

The structure of GG has been confirmed by X-ray crystallography. GG crystallises from water as the tetrahydrate in

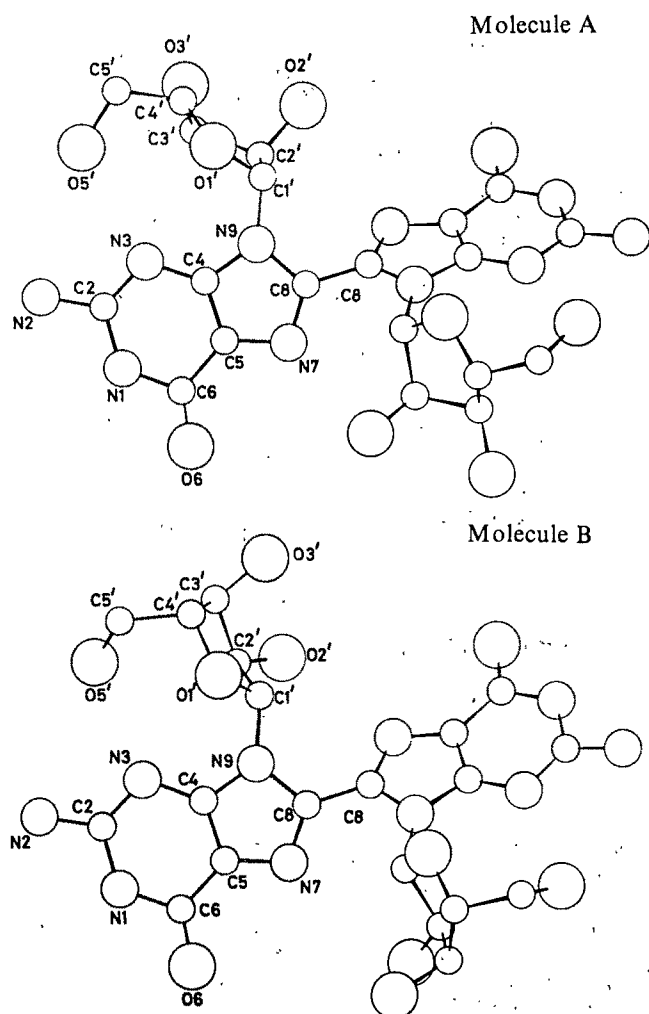


Fig. 2 The two independent molecules of GG in the crystalline tetrahydrate viewed perpendicular to the base of one half of the molecule in each case.

the form of colourless needles elongated along *b*, space group *C2* with $a = 26.69(1)$, $b = 8.425(5)$, $c = 12.286(7)$ Å, $\beta = 104.9(2)^\circ$. A total of 1,740 unique reflections were collected on a Philips PW1100 four-circle diffractometer of which 1,071 had $I(\text{rel}) > 2\sigma I(\text{rel})$ and were considered observed. The structure was solved by a multi-solution tangent refinement using the SHELX programme and refined, with all atoms treated isotropically, to a final *R* of 0.068.

There are two independent half-molecules in the crystallographic asymmetric unit, with the other halves generated by a twofold axis which runs through the centre of the bond joining the C8 atoms of the purine rings. Figure 2 shows the two independent molecules viewed perpendicular to the base of one half of the molecule in each case.

Table 1 Ultraviolet absorption and fluorescence characteristics of (8 \rightarrow 8) coupled purine nucleosides at pH 7.0

Compound	Ultraviolet absorption		Fluorescence	
	λ_{max} (nm)	ϵ ($\times 10^{-3}$)	$\lambda_{\text{max}}^{\text{ex}}$ (nm)	$\lambda_{\text{max}}^{\text{em}}$ (nm)
GG	278	24.4	330	405
	322	21.5		
AG	280	13.6	331	415
	321	18.0		
IG	278	—	326	415
	315	—		

The ultraviolet spectra were recorded using a Cary 118 instrument. The fluorescence spectra, which are uncorrected, were recorded using a Perkin-Elmer MPF-2A spectrofluorimeter.

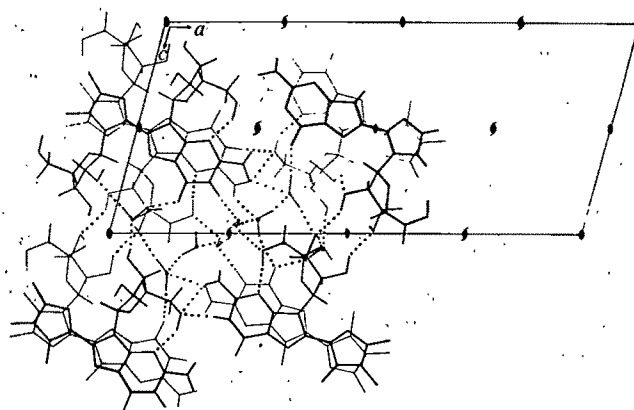


Fig. 3 The structure of GG tetrahydrate viewed down the *b* axis. Hydrogen bonds are indicated by dotted lines.

The torsion angles χ_{CN} ($\text{O1}'\text{-C1}'\text{-N9-C4}$) (ref. 5) are 35.7° and 47.6° for molecules A and B respectively. The orientation about the glycosidic bonds is thus *syn* for both molecules, as expected for nucleosides with bulky substituents at C8 (ref. 6). In addition both A and B molecules contain *intra* molecular $\text{O5}'\text{-H} \cdots \text{N3}$ hydrogen bonds which provide additional stabilisation for the *syn* conformation.

The ribose ring conformations of the two independent molecules differ considerably. In molecule A they show a C3' *endo* puckering distorted towards a C4' *exo* conformation, with a pseudorotation parameter⁷ $P = 28.6^\circ$ (2T_4). The ribose rings of molecule B show C2' *endo* puckering distorted towards C1' *exo* conformations, with $P = 156.2^\circ$ (2T_1). The conformation about the C4'-C5' bonds in both molecules is *gauche-gauche*. The torsion angles $\text{O5}'\text{-C5}'\text{-C4}'\text{-C3}'$, $\text{O5}'\text{-C5}'\text{-C4}'\text{-O1}'$ are 59.0° , -56.8° and 47.6° , -69.3° for molecules A and B respectively. Within the individual molecules the bases are twisted about the C8-C8 bond, with the angles between least squares planes of the separate bases equal to 53.3° (molecule A) and 41.8° (molecule B). The structure consists of continuous columns of stacked bases. The interplanar distances between successive bases, measured at the C8-C8 bonds, are alternately 4.86 and 3.56 Å. The structure is extensively hydrogen bonded. The water molecules form a network of hydrogen bonds concentrated in columns about alternate twofold screw axes as shown in Fig. 3.

This work was supported by the SRC.

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reviews

Climatic shifts

John Gribbin

Climates of Hunger: Mankind and the World's Changing Weather. By Reid A. Bryson and Thomas J. Murray. Pp. xv + 171. (American University Publishers Group: London, 1977.) £6.75.

PROFESSOR BRYSON is perhaps the leading proponent of the view that anthropogenic pollution of the atmosphere is hastening the world into a pronounced cooling—at least, a little Ice Age—through the effect of aerosol particles blocking out some of the heat from the Sun. Thomas Murray is a science writer who has worked with the University of Wisconsin-Madison, where Bryson's Institute for Environmental Sciences is based. Working in harness, they have produced a book about the possible hazard to world food supplies of a significant global cooling, but one which is aimed very clearly at the general reader and the non-scientist, including, perhaps, those with some political influence on the world food situation.

The result is a very easy, even glib, read, which is strong on anecdotal material about the impact of past climatic shifts on human societies, but is weak on mechanisms of climatic change and which has no real scientific depth. It was clearly written for a purpose, to spread the message of Bryson's ideas beyond his peer group of climatologists, and even beyond the scientific ranks of those who, for example, regularly read *Nature*. It does the intended job well, as indicated by the selection of the book as a US Book-of-the-Month Club choice, but this means that someone who is a regular reader of *Nature* need feel no compunction to rush out and buy the book, but can safely wait for a paperback without missing any significant new contribution from Bryson.

This is very much a Bryson's eye-view; the casual reader will not get a balanced picture of the current climatic debate, but that is of far less importance than interesting such a reader in the debate in the first place. The balance, or lack of balance, is indicated by the reference list, where no less than 25% of the 80-odd works cited are authored or co-authored by Bryson.

The most notable omission from that reference list is Stephen Schneider's *The Genesis Strategy* (reviewed in *Nature* 264, 137; 11 November 1976)

which is still, to my mind, the best introduction to climatic change for the non-specialist. The gaps in the text of this new book which make it unsuitable as introductory reading in an academic context are typified by the devotion of only four short paragraphs to solar changes, within a mere nine-page chapter "How Climate Changes".

But the importance of all books of this kind is that they draw attention to the possibility of irregular fluctuations in natural forces which can affect world food production. The fact that the world could feed twice the present population, but yet people are starving today, indicates that something is wrong with the overall production and distribution of food. When reserve stocks are deliberately kept low to

maintain prices on world markets, the prospect of a run of bad harvests is grim, whether or not you subscribe to Bryson's detailed views on why such a pattern of events may take place. Any book which may encourage more sensible husbandry must be welcomed; I hope, however, that the likely success of this little book in its evangelical role will encourage Professor Bryson to provide us with something more technical which we can get our teeth into. The importance of his ideas certainly justifies the devotion of a major text to their display in the academic market place. □

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Dissipative structures

Self-Organisation in Non-Equilibrium Systems: From Dissipative Structure to Order through Fluctuations. By G. Nicolis and I. Prigogine. Pp. xiii + 492. (Wiley-Interscience: New York and London, 1977.) £20; \$34.

THE central key word of the monograph by Nicolis and Prigogine is *Dissipative Structure*. This concept already seems to have become a paradigm, at least in theoretical biology. What is a dissipative structure? The formation of flow structures in physical systems under certain conditions is commonly known. The most frequently quoted dynamic pattern of this type is the Bénard honeycomb-like structure of a liquid heated from below; this phenomenon results from the coupling of heat flow and local convection currents. A further impressive example for spatial and temporal order in a flow structure is the Belousov-Zhabotinski reaction; here, it is the interplay between chemical reactions and diffusion that creates periodic patterns in time and space. Such flow structures exist only within certain boundary conditions and survive only on energy input dissipated in the maintenance of the flow pattern.

Such close similarities exist between the behaviour of a dissipative structure and the properties of living organisms that it is intriguing to investigate living

organisations in terms of flow interactions. The 'bioflows' are, of course, not heat flows or rarely convection flows, but the rates of coupled biochemical reactions and diffusion processes in the open systems of biology. It does seem very exciting to find out whether the various flow patterns observed in chemistry and biology are based on one and the same fundamental principle of coupling between chemical reactivity and diffusional propagation.

The book by Nicolis and Prigogine presents a concise introduction to theoretical foundations and applications of new concepts and offers to approach flow phenomena programmatically in the framework of a general dynamic theory. This introduction to the theory of dissipative structures aims at a better understanding of the evolution and survival of self-organising systems in physics and biology.

Dissipative structures have been recognised to exist only far from equilibrium where the relationships between flows and driving forces are non-linear. Thus, classical non-equilibrium thermodynamics (relating flows and potential gradients linearly) do not provide adequate tools to describe dissipative flow structures. Among the new concepts introduced by the Brussels school is the definition of a local potential which generalises the familiar chemi-

cal potential for an application to encompass non-equilibrium states. The behaviour of fluctuations in the local thermodynamic properties now permits a distinction to be made between stable and unstable states. If the potential gradients are small, each deviation from the steady-state rapidly decays to the stationary level. When the gradients become increasingly larger, however, then the fluctuations can increase beyond the instability point where a new dissipative flow structure can be established: development of order, that is, self-organisation through fluctuations.

A useful criterion for flow stability is introduced by the "excess entropy production". This entropy function is positive for stable or steady-states and negative for the evolution of a dissipative structure. As expressed in the introduction of the monograph, the authors intend to present a unified formulation for apparently very different self-organisation phenomena in complex systems. It does seem very appealing to consider such different manifestations of "flow order" as living beings, the generation of coherent light by a laser, the emergence of spatial and temporal pattern of activity in chemical kinetics and in fluid dynamics, or the functioning of an animal ecosystem or of a human society as dissipative self-organisation in far-from-equilibrium conditions. The book is therefore conceived as a basic guide.

In part one, the thermodynamic background for the description of flow phenomena is outlined and the basic principles of non-equilibrium thermodynamics are introduced, with special

emphasis on chemical reactions coupled to transport processes such as diffusion. Part two covers mathematical aspects of self-organisation using simple autocatalysis and other feedback models. Part three deals with stochastic methods for the description of fluctuations and diffusional processes. In part four, control mechanisms in chemical and biological systems are discussed: regulatory processes at the subcellular and cellular level, cellular differentiation, and pattern formation. Part five is devoted to evolution and population dynamics and touches on, among various examples, reaction networks of biopolymer self-organisation, the thermodynamics of ecosystems, neural and immune networks, social systems and epistemological questions.

The book covers an impressively broad field showing parallels and similarities in the substructure of various complex dynamic phenomena in nature. It remains true, however, that it is dangerous to project the concepts of one discipline to another field and to use analogies instead of careful analysis of the particular problem under investigation. But this enthusiastically written book by Nicolis and Prigogine—although not very easy to read for non-theoreticians—clearly elaborates to what extent structural parallels can be recognised and how general notions can be applied to very different dynamic structures in order to gain a better understanding of self-organisation.

E. Neumann

E. Neumann is a member of the staff at the Max-Planck-Institut für Biochemie, München, FRG.

Primate studies and methodology

Primate Ecology: Studies of Feeding and Ranging Behaviour in Lemurs, Monkeys and Apes. Edited by T. H. Clutton-Brock. Pp. xxii+631. (Academic: London, New York and San Francisco, 1977.) £25; \$41.

STUDIES of seventeen primate species make up the bulk of this important book; review chapters and appendices on methodology the remainder. Few population-orientated primate studies have been attempted and the papers concentrate on the detailed sampling of individual behaviour in one or two social groups. Interspecific, interpopulation, intergroup and interindividual differences in feeding and ranging behaviour are all discussed, as are diurnal and seasonal changes in diet. The fine level of analysis used by most

authors may prove tiresome to the non-specialist but it is fully justified. Most papers have adequate summaries.

The studies spring from a decade's speculation on the relationship between ecology and social organisation, and most contributors develop evolutionary hypotheses about the adaptive significance of behaviour. The experimental manipulation of resources is largely out of the question and the usefulness of the conclusions will rest largely on their applicability to different populations or species. Review chapters by the editor and Harvey, consider intra- and interspecific variation in ecology and behaviour.

The diets of all seventeen species are described. Clutton-Brock reviews methods for sampling feeding, Harvey reviews the measurement of dietetic diversity, and Hladik describes field methods for collecting and processing food samples. These chapters, together with each authors' attention to methodology, will make this a standard reference volume. The chemical composition of food is given by several

authors and some interesting conclusions follow. Calculations by A. Goodall confirm that a completely herbivorous gorilla could obtain ample energy and protein; but Fossey and Harcourt consider that gorillas eat grubs to get vitamin B₁₂. The consumption of ants and termites by chimps is shown by Hladik to compensate for essential amino acids lacking in plant foods. The balancing of diet during a day, between protein-rich buds and leaves and carbohydrate-rich flowers and fruit, is shown for Howlers (Smith), Orang-utans (Rodman), Siamang (Chivers) and Chimpanzees (Hladik). Primatologists are now paying attention to potentially toxic secondary compounds in food plants. Hladik argues, however, that though these compounds may protect plants against non-specialised frugivores such as macaques, they provide little protection against the specialised leaf eaters such as *Presbytis senex* in which bacterial decomposition is assured before intestinal absorption.

Another major theme discussed is the interaction between diet and social organisation. Primatologists now study the forest flora meticulously. Food trees are mapped, leaf and fruit abundance recorded and the primates' daily movements are related to changes in food availability. Some general patterns are emerging. Sussman found that the folivorous *Lemmus fulvus* lives in small groups with small ranges and uses few food species, in contrast to the more frugivorous *L. catta*. Parallel interspecific differences are seen in *Presbytis* and *Colobus*. At a finer level, the relationship between food availability and subgroup formation is reported for spider monkeys by Klein and Klein, and for chimpanzees by Wrangham. In contrast to earlier speculation, Wrangham found no evidence that the frequent dispersal and reformation of chimpanzee subgroups increases food-searching efficiency. The so called "food calls" (loud calls given by males on finding large food sources) are given most frequently when food is plentiful, not when it is scarce. Wrangham considers that a calling male "puts up" with increased feeding competition because some of the attracted animals will be potential mates.

This is a book for every library. It would be splendid if some of those reading it set out to make the population-orientated ecological studies (for example, of population dynamics) which are absent from the present volume. In the more open-country primates, these will not be as difficult as the editor implies in his introduction.

John M. Deag

John M. Deag is Lecturer in Zoology at the University of Edinburgh, Scotland.

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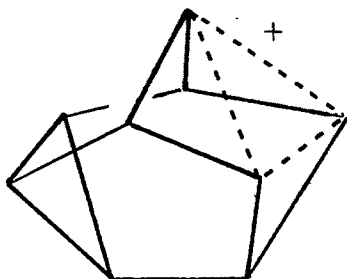
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Non-classical carbonium ions?

The Non-Classical Ion Problem. By Herbert C. Brown, with comments by Paul von R. Schleyer. Pp. 301. (Plenum: New York, 1977.) \$29.95.

THE non-classical carbonium ion is a formulation for an organic cation, no one structure for which, written with normal two-electron bonds, adequately describes the properties observed. One carbon atom (or more) is assigned a coordination number exceeding four, and some of the bonds are considered partial, and dotted; precise definition presents some difficulty. Proposed once in 1939, and freely in the 1950s, this concept came under rigorous scrutiny by the Purdue school, who agreed that it was acceptable *a priori* but asked whether it was needed, or whether the rapid interconversion of classical structures would explain observations more economically. An unusually vigorous controversy ensued, in which organic chemists not themselves involved have taken the opposed views, either that it was a sterile question attracting attention better directed

elsewhere, or that it represented an important stage in the development of chemical theory, like the debate on tautomerism in about 1900. Documents published before mid-1965 were issued in reduced format, inter-related by a commentary by P. D. Bartlett, in 1966.



Now we have a full statement of the case for the prosecution, written in 1976-77 by H. C. Brown, with shorter comments by P. von R. Schleyer as advocate for the defence. Whatever else is said, the idea has encouraged non-classical formats in publication.

The subject is important, and this book is an excellent summary of the field. Much progress has been made. Brown's concept of equilibrating classical ions is now proved for many sys-

tems, and some early views on steric effects are now undisputed. On the other hand, the phenonium ion, attacked by Brown in 1962, is now accepted, along with the knowledge that solvolysis reactions generally involve nucleophilic assistance from solvent (thanks largely to Schleyer). At last Brown can write of "Coate's cation" (see Figure): "the proposal that solvolysis proceeds through the non-classical ion appears reasonable"; so the way is now open to agreement on effects to be expected from substituent and solvent changes in such species.

The debate is not over; even where Brown and Schleyer agree, the reader may decide to differ. He will, however, find this book readable and cogent throughout, and he will be able to move about it, checking points with the aid of a good index. He will be repeatedly reminded of the fundamental aims of organic chemistry. He will reach his own conclusions, and will probably end by deciding that the great controversy was worthwhile, after all.

M. C. Whiting

M. C. Whiting is Professor of Organic Chemistry at the University of Bristol, UK.

Prostaglandin synthesis

The Synthesis of Prostaglandins. By A. Mitra. Pp. 444. (Wiley: London and New York, 1977.) £16.90; \$28.60.

THE field of prostaglandin (PG) synthesis has received the attention of many organic chemists over the past decade, and as a result much novel and intriguing chemistry has been developed. Abhijit Mitra has set out to bring together, in this single review, the various synthetic strategies that have been used up to the middle of 1977, and to describe many intimate details of their execution.

Taken in order, the book begins with a brief, and at times misleading, description of PG biosynthesis; the unlikely implication that thromboxane A_2 is partially converted to PGE_2 and $PGF_{2\alpha}$ *in vivo* is an example. This minor blemish apart, chapters 2-15 describe the numerous approaches to the PGE and PGF systems in a very detailed manner. The individual syntheses are classified by the particular strategy used, such as that involving 1,4-addition to substituted cyclopentenones as the "fundamental" step, and that depending on the well known Corey lactone. This system works well, and renders the information readily accessible; however, it may be that a classification based on the historical development of such ingenious synthetic chemistry would have afforded the browsing reader with

greater aesthetic rewards. Rather obscurely, one version of the synthesis of prostacyclin from $PGF_{2\alpha}$ lies buried in the chapter devoted to the Corey approach, together with a description, without references, of some prostacyclin analogues.

The remainder of the book is devoted to the synthesis of thromboxane B_2 , PGA_2 , PGC_2 , and finally, various PG analogues. This last chapter covers not only the very close relatives, such as 15-methyl- PGE_2 , but also some of the numerous heterocyclic and hetero-chain analogues that have been prepared; however, the complete lack of patent coverage, which is a significant drawback of the book as a work of reference, is very noticeable in this section. There are also some minor omissions from the published literature, such as the Upjohn *ent*-PGs, and the *seco*-PG analogues of Merck.

In summary, the book contains a very useful, if somewhat non-critical, review of the literature on PG synthesis, the coverage being generally quite comprehensive. For the synthetic chemist working in the field, the book will be an invaluable aid, with its clear and concise diagrammatic presentation and thorough indexing. For the non-specialist reader, there is described much elegant molecular architecture to be perused and enjoyed.

C. J. Harris

C. J. Harris is a research chemist at the Wellcome Foundation Research Laboratories, Beckenham, UK.

Truelove Lowland, Devon Island, Canada: A High Arctic Ecosystem

edited by L.C. Bliss, PhD

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Analysis of cell organisation

International Cell Biology, 1976-1977. Edited by B. R. Brinkley and K. R. Porter. Pp. 694. (Rockefeller University Press: New York, 1977.) \$30.

THIS volume derives from the First International Congress on Cell Biology, held in Boston in 1976. It contains shortened versions of sixty or so invited contributions to twenty-two miniature symposia which formed part of that congress. Since it deals at some length with a wide range of topics which were judged to be of particular interest at that time, the editors invite us to regard it as "a historic document of cell biology in 1976", which "future scholars should find a valuable source of information". The claim is, of course, extravagant, but let us take it at face value. What would a future scholar, perusing these pages, discover about cell biology in the mid-1970s?

The most striking impression, I believe, would be of the extent to which the analysis of cellular structure and process is being carried down to the molecular level. Cell and molecular biology are meeting and fusing. In this connection it is, I think, not out of place to quote some words of E. O. Wilson about the nature of the relationship that exists between contiguous levels of analysis in science. In a recent article he writes: "For every discipline in its early stages of development there exists an antidiscipline. For many-body physics, particle physics; for chemistry, many-body physics; for molecular biology, chemistry; for cellular biology, molecular biology; and so forth. With the word *antidiscipline*, I wish to emphasise the special adversary relationship that exists initially between the studies of adjacent levels of organisation. This relationship is also creative, and with the passage of a great deal of time it becomes fully complementary."

I believe that there can be no doubt that, in these terms, after a period of mutual indifference, if not antipathy, the creative, complementary phase of the relationship between cell and molecular biology is at hand. Indeed, the evidence for this collected here is the most impressive aspect of the present volume. Not in every article, but in a high proportion, we see that the molecular basis of cell organisation is being uncovered with considerable speed and success.

As one contributor writes, in connection with chloroplasts: "With the investigative strategies now available, nothing but the discipline of experi-

mentation is needed to reveal the interactions between individual thylakoid proteins and between proteins and lipids. The future challenge is to unravel the intricate mechanism of the assembly of chloroplast membranes from their diverse components and to explain how this assembly is controlled by the complex interaction of the chloroplast and nuclear genomes." The passage is typical, unexceptional. Note, too, that "nothing but the discipline of experimentation" is seemingly needed. This is another feature of the subject as displayed here. Cell biologists now have at their disposal a prodigious array of analytical techniques and an almost unlimited territory to explore with them. New ideas are not, for the moment, held to be the prime necessity: it is tacitly assumed that advances are assured, and will come primarily by making observations and doing experiments. One author, for example, asserts quite confidently that in the next decade with the new methods of somatic cell genetics it will be possible to map over a thousand human genes.

There are almost no theoretical articles in this volume and scarcely anyone seeks principles or generalisations that might encompass more than his own immediate area of enquiry. It is an interesting phase of the subject, bewildering and stimulating in the multiplicity of new data.

This is better than most such volumes and contains at least a few excellent papers. It will, however, have only a rather limited useful life. There is little that one cannot, with moderate diligence, find elsewhere. Some articles are confined to reporting more or less new facts; others, generally much more useful, provide a broader review of their subject. Many of the articles would have been better if they could have been longer. The symposium on secretion in animal cells achieves a degree of coherence that would have been welcome elsewhere. The editing and production are first-rate.

A. V. Grimstone

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Laser spectroscopy

Non-Linear Laser Spectroscopy. By V. S. Letokhov and V. P. Chebotayev. Pp. 466. (Springer: Berlin and New York, 1977.) DM68; \$30.

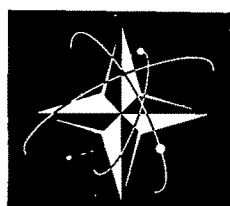
THIS is a timely and authoritative monograph on the non-linear interaction of laser radiation fields with Doppler-broadened atomic and molecular transitions by the leaders of the distinguished Russian school of laser spectroscopists. The ability of coherent, intense optical fields to saturate a transition, to interact only with a narrow range of molecular velocities, to enhance multiphoton processes, all these and many more have opened up a qualitatively new sort of spectroscopy. Experimental resolution has often increased by several orders of magnitude and we can now observe effects unimaginable a decade or so ago. This book attempts to describe the theory and experimental methods of all those features which can be grouped loosely under the heading of "non-linear laser Doppler-free spectroscopy". The authors develop the density-matrix equations of motion for single and multiphoton absorption and apply them to a description of saturated absorption with both counter-propagating and colinear probe and saturating beams. This is followed by a discussion of Doppler-free two-photon transitions using rather

simpler theory, and a treatment of coupled three-level systems (including Raman processes). This theoretical framework is then applied to specific problems in atomic and molecular spectroscopy, with attention being given to limiting factors on resolution and on stabilisation in precision "standards" experiments.

The book is written in a rather strange grammatical style afflicted by an over-literal translation (apparently by the authors) of the Russian original. This is certainly no worse than the style of the AIP translation journals in which a great part of the original material first appeared in the West, and detracts only very slightly from the value of the book. It is a pity the authors did not include a more complete discussion of the role of the dynamic Stark effect on saturated resonance fluorescence in strong fields, a subject of great interest for the past four years or so. More generally there is little or nothing on quantum aspects of the electromagnetic field, with an emphasis on semiclassical descriptions. The typeface is the unpleasant typewriter form we are forced to accept in these depressed days, but in return the price is compensatingly realistic. In spite of these defects, the book will be of great value to laser spectroscopists and to atomic physicists contemplating using the new laser techniques.

Peter Knight

Peter Knight is Jubilee Research Fellow in the Department of Physics at Royal Holloway College, University of London, UK.



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APPOINTMENTS VACANT

UNIVERSITY OF SURREY DEPARTMENT OF BIOCHEMISTRY LABORATORY SUPERINTENDENT

Applications are invited for the post of Laboratory Superintendent in the Department of Biochemistry.

The Department is multidisciplinary with emphasis on medicine and the pathological sciences and is very active in research. The person appointed (male or female) will be in charge of a team of about 25 technical staff and will be directly responsible to the Head of Department for the organisation and efficient operation of the laboratories and technical services for both teaching and research.

Applicants must have a relevant qualification; with wide experience in biochemical laboratory practice and techniques and should have previously held a position of responsibility in laboratory management.

The salary will be on the Senior Experimental Officer scale: £5,219 to £6,655 per annum, the commencing point according to qualifications and experience.

Applications, in the form of a curriculum vitae, giving full details of age, qualifications and experience, and the names and addresses of two referees, should be sent to the Assistant Secretary (Personnel), University of Surrey, Guildford, Surrey GU2 5XH, by:—March 16, 1978 and from whom further particulars of the post can be obtained: Telephone Guildford 71281, Ext. 452. 7505(A)

PROFESSOR, SENIOR LEVEL PHYSICAL/BIOPHYSICAL CHEMISTRY UNIVERSITY OF CALIFORNIA RIVERSIDE

The Department of Chemistry has a position for a distinguished person as Professor of Chemistry, effective July 1978, or later. Candidates should be internationally recognised for current research in the forefront of physical chemistry. Preference will be given to biophysical candidates who complement present research offerings (there is a potential for joint appointment in Biochemistry, if appropriate). Candidates must have demonstrated ability to make significant contributions to the undergraduate and graduate instructional programmes. We are especially interested in receiving applications from women and minority group candidates. Requests for confidentiality of applications will be honoured. Submit curriculum vitae and list of publications to: M. Gibian, Chairman, Search Committee.

Department of Chemistry
University of California
Riverside, CA 92521

An Equal Opportunity/Affirmative Action Employer. 1495(A)

MICHIGAN STATE UNIVERSITY POSTDOCTORAL RESEARCH ASSOCIATES IN ENVIRONMENTAL CARCINOGENESIS

Openings for research associates with strong background in molecular biology, biochemistry, microbiology, or biophysics to study the relationship between DNA repair, mutagenesis, and oncogenic transformation of human cells in culture by physical, chemical, and viral carcinogenic agents. Send application, including curriculum vitae, reprints, and letters of recommendation to: Dr V. M. Maher or Dr J. J. McCormick, Co-Directors, Carcinogenesis Laboratory, COM, Michigan State University, E. Lansing, Michigan 48824. 1536(A)

SECOND AIN SHAMS MEDICAL CONGRESS FACULTY OF MEDICINE Cairo

October 21–25, 1978

Ain Shams Faculty of Medicine announces its "Second Ain Shams Medical Congress" to be held in Cairo from October 21–25, 1978. This congress will include 3 days for all branches of medicine and 2 days on a special subject which will be "Immunology".

Application forms of detailed informations will be sent on request.

Please write to:

Professor Aly Abdel-Fattah,
Secretary General
Ain Shams Faculty of Medicine
Abbassia, Cairo.
P.O. Box 38, Abbassia. 1483(C)

INSTITUTE OF CELLULAR AND MOLECULAR PATHOLOGY

DEPARTMENT OF MOLECULAR BIOLOGY

Applications are invited from candidates, possessing a Ph.D. in molecular biology, molecular genetics or biochemistry, for postdoctoral or junior faculty research positions. Candidates should be interested in gene cloning and recombinant DNA research and experience in nucleic acid technology would be an advantage. The ability to speak French is not essential. Positions are available for three years.

Send curriculum vitae, list of publications and the names of three referees to Dr John Davison, Department of Molecular Biology, ICP, Avenue Hippocrate 75, B-1200 Brussels, Belgium. 1550(A)

IMPORTANT RESEARCH DIVISION OF AN INTERNATIONAL PHARMACEUTICAL GROUP

located in PARIS is offering an **EXCELLENT CAREER
OPPORTUNITY** for a

FIRST CLASS SENIOR CARDIOVASCULAR PHARMACOLOGIST

to join his **BIOLOGY DEPARTMENT**

Preference will be given to individuals with the following qualifications:

- M.D., Ph.D. degree or equivalent.
- relevant postdoctoral or industrial experience (3 to 5 years).
- significant basic research in the field of hypertension, mainly on the central and peripheral mechanisms involved in the regulation of the blood pressure.
- good attitude towards team work.
- good potential for managing people.

The applicant will be responsible to the Director of Biology.

An excellent salary and comprehensive benefit programme accompany this position.

Please send curriculum vitae to Box No. 1272(A), Nature Magazine, c/o Macmillan Journals Ltd., 4 Little Essex Street, London WC2R 3LF.

1272(A)

South African Astronomical Observatory Research Posts

Two temporary (3 year) or permanent research posts are available at the South African Astronomical Observatory which is financed jointly by the Council for Scientific and Industrial Research (South Africa) and the Science Research Council (United Kingdom). The astronomy staff of the SAAO is based in Cape Town where the main offices, etc. are situated. Most observing facilities are at Sutherland (Cape Province) where the SAAO has 1.9m; 1.0m; 0.75m and 0.5m reflectors equipped with a range of modern instrumentation.

Preference will be given to applicants with research experience and a Ph.D. degree or equivalent, and whose main interest is observational astronomy.

Salary will be determined by qualifications and experience. Assistance will be given with transfer expenses.

Fringe benefits include 39 days leave per annum (maximum), generous sick leave, and medical and pension schemes.

Enquiries should be directed to the Office of the Scientific Counsellor,

South African Embassy:

278 High Holborn, London WC1V 7HE (Tel. 01-242 1766)

59, quai d'Orsay 75007 Paris (Tel. 555-92-37)

P.O. Box 12-1366 Tehran (Tel. 62-1835/6/7)

5300 Bonn 2, Auf der Hostert 3 (Tel. 36 3047/48)

3rd Floor, 2555 M Street N.W. Washington D.C. 20037

(Tel. 833-3860)

1453(A)

CENTRE DE RECHERCHE MERRELL INTERNATIONAL— STRASBOURG (France)

A position (Ph.D.) level is available for a
**SENIOR ANALYTICAL
BIOCHEMIST**

The work involves participation in ongoing biochemical programmes and initiating and running pharmacokinetic and metabolic studies in animals and in man.

Interested persons should send their curriculum vitae to the Director, Dr Jan Koch-Weser, Centre de Recherche Merrell International, 16 rue d'Ankara, 67084 Strasbourg, France. 1478(A)

UNIVERSITY OF OXFORD CLARENDON LABORATORY RESEARCH ASSOCIATE

Applications are invited for the post of research associate in the field of beam foil spectroscopy. The work will include measurements of fine structures and Lamb shifts by the application of newly developed spectroscopic techniques to highly ionised atoms produced by the Nuclear Physics Laboratory's Tandem van de Graaff accelerators.

Applicants should have had some years' experience in experimental atomic physics research, should preferably hold or be about to obtain a doctoral degree. The appointment, for two years in the first instance, will be on the scale £2,904 to £4,811 (under review).

Applications, giving a curriculum vitae and the names of two referees, should be sent to Dr J. D. Silver, Clarendon Laboratory, Parks Road, Oxford OX1 3PU, England, by March 31, 1978. 1493(A)

PHYSIOLOGIST

required by the Medical Research Council Cyclotron Unit to join the Biology Section to work with normal tissues using experimental animals. Studies will include effects of both neutrons and X rays and certain adjuvant treatments used in radiotherapy. Experience in neurophysiology and/or pharmacology would be an advantage. Post vacant mid-April. The applicant must have a First or Upper Second Class degree in the appropriate subject and at least 3 years postgraduate research experience or a degree in medical or veterinary sciences.

Salary, according to age and experience, in the range £3,780 to £5,660 (inclusive of pay supplements and London Weighting) plus 6 weeks annual leave.

Please write, giving qualifications and curriculum vitae, to:

Miss A. Pires,
M.R.C. Cyclotron Unit,
HAMMERSMITH HOSPITAL,
Ducane Road, London W12 0HS.
Tel: 01-743 4594, ext. 103
(Ref: P/2.)

1555(A)

THE QUEEN'S UNIVERSITY OF BELFAST DEPARTMENT OF PHYSIOLOGY

Two lectureships in the department of Physiology are available from October 1, 1978 or such other dates as may be arranged, and the successful applicants will assist in teaching medical, dental and science undergraduates.

LECTURESHP

On lecturer, if medically qualified, will be appointed on the scale (under review): £4,190 to £6,655. A salary on a sessional basis may also be paid by the Eastern Health and Social Services Board for hospital services undertaken in addition to University duties. If not medically qualified the lecturer will be appointed on the scale (under review): £3,333 to £6,655. Initial placing on above scales is dependent upon qualifications and experience.

LECTURESHP ON PROBATION

Appointment to the second lectureship is subject to a period of probation of up to three years in duration and might be of particular interest to junior doctors working for a higher degree in medicine or surgery by thesis or by examination. It will be made initially at one of the points £3,975, £4,190, £4,403, £4,607, or for non-medical applicants at one of the points £3,333, £3,547, £3,761, on the scale (under review) rising to £6,655.

Assistance with removal expenses is available, and contributory pension rights under the FSSU/USS apply. Closing date: March 22, 1978. Further particulars may be obtained from the Personnel Officer, The Queen's University of Belfast, BT7 1NN, Northern Ireland. (Please quote Ref. 78/N.) 1521(A)

THE UNIVERSITY OF LEEDS DEPARTMENT OF BIOCHEMISTRY

Applications are invited for the post of DEMONSTRATOR in the above Department, available from May 1, 1978. The successful candidate will be expected to assist in teaching biochemistry to science and to medical students and to carry out research in association with existing members of the academic staff. The appointment is tenable until September 30, 1979.

Salary on the scale £2,904 to £4,811 of the IB Scale for Other Related Staff.

Application forms and further particulars may be obtained from the Registrar, The University, Leeds LS2 9JT, quoting reference number 83/11. Closing date for applications March 17, 1978. 1492(A)

UNIVERSITY OF LONDON INSTITUTE OF PSYCHIATRY RESEARCH WORKER (Scale IA)

required for an investigation of factors controlling the enzymic N-methylation of tryptamines in rodent and human tissues. Postdoctoral experience in enzyme purification and gas chromatography desirable, but not essential. This post is supported by M.R.C. grant for three years.

Salary in range £3,975 to £4,403 per annum, plus £450 London Weighting.

Application forms and further details available from the Secretary, Institute of Psychiatry, De Crespigny Park, Denmark Hill, London SE5 8AF, quoting ref RR/N. Application forms should be completed and returned by 10 March 1978. 1562(A)

UNIVERSITY OF STRATHCLYDE

Applications are invited from post-doctoral experimental physicists for a LECTURESHP in the DEPARTMENT OF NATURAL PHILOSOPHY. Applicants should have practical knowledge and experience of high-voltage technology and an interest in charged particle beams and/or plasma physics. The successful candidate will be required to participate in general undergraduate and postgraduate teaching in physics and in the research interests of the Department.

Salary scale £3,333 to £6,644 per annum (under review) with placing according to qualifications and experience. Superannuation benefit.

Application forms and further particulars (quoting 12/78) and enclosing a self-addressed envelope (9in. by 4in.), may be obtained from the Academic Appointments Officer, University of Strathclyde, Royal College Building, 204 George Street, Glasgow G1 1XW with whom applications should be lodged by March 13, 1978. 1515(A)

UNIVERSITY OF EDINBURGH DEPARTMENT OF MOLECULAR BIOLOGY

Applications are invited for an M.R.C. postdoctoral fellowship to work on the molecular relationships of plasmids, principally those carried by enterobacteria and *Pseudomonas* species from soil and clinical sources. A variety of biochemical and genetical techniques will be involved. These will include DNA-DNA hybridisation and probably heteroduplex analysis and the generation of recombinant plasmids both *in vivo* and *in vitro*. The appointment is for three years from April 1978 or when convenient thereafter. The starting salary will be between £3,333 and £4,190 p.a. as appropriate.

Applications with curriculum vitae and the names and addresses of two referees should be sent to Dr P. M. A. Broda, Department of Molecular Biology, King's Buildings, Mayfield Road, Edinburgh, EH9 3JR. Please quote Reference 5001. 1436(A)

YALE UNIVERSITY SCHOOL OF MEDICINE NEUROBIOLOGIST

The Department of Physiology is seeking a neurobiologist at the Assistant Professor level. Preference will be given to individuals whose work relates to the function of the central nervous system. The position permits considerable time for research; opportunities exist for graduate and medical student teaching.

Please send curriculum vitae, bibliography and the names and addresses of three references to:

CHAIRMAN
Neurobiologist Search Committee
Department of Physiology
Yale University School of Medicine
333 Cedar Street
New Haven, Conn. 06510

Applications from women and members of minority groups are encouraged. An Equal Opportunity/Affirmative Action Employer.

Applications should be received before March 30, 1978. 1419(A)

UNIVERSITY OF CAMBRIDGE DEPARTMENT OF PATHOLOGY RESEARCH TECHNICIAN

required for work on project involving cellular immunology in the Division of Immunology, Department of Pathology. Qualifications: H.N.C. or equivalent. Experience of laboratory work in immunology, cell biology or biochemistry desirable. Salary range £2,695 to £3,087. Applications in writing to The Superintendent, Department of Pathology, Tennis Court Road, Cambridge CB2 1QP. 1530(A)

UNIVERSITY OF SYDNEY
PROFESSIONAL
ASSISTANTS—
GRADES I and II or
RESEARCH FELLOW
SCHOOL OF PHYSICS
(SOLAR ENERGY GROUP)

The N.S.W. State Government grant for solar energy research within the School of Physics has made possible an expansion of the research effort on selective surfaces and advanced solar collectors. Desirably the positions will be filled at the post-doctoral or equivalent experience level by experimental physicists where experience in solid state or optics would be an advantage. Appointees will be responsible to the leader of the solar energy research group for specific parts of the solar energy projects.

All appointments will be to December 31, 1978 in the first instance, but may be renewed.

Salary Range: Professional Officers \$A10,264 to \$A15,703 p.a. Research Fellow \$A14,851 to \$A19,551 p.a.

Applications including details of qualifications, experience and names of two referees by March 31, 1978, to Professor H. Messel, University of Sydney, N.S.W. 2006, Australia, from whom further information is available. 1514(A)

MEDICAL RESEARCH
COUNCIL
NATIONAL INSTITUTE FOR
MEDICAL RESEARCH
BIOCHEMIST/
MICROBIOLOGIST

A postdoctoral biochemist or microbiologist aged up to 29 years is required by the LABORATORY FOR LEPROSY AND MYCOBACTERIAL RESEARCH to study aspects of the intermediary metabolism of *Mycobacterium leprae* (the human leprosy bacillus). Quantities of bacteria sufficient to undertake such studies are available in the Laboratory for the first time.

The appointment will be for up to three years and will be in the present salary range £3,995 to £5,219 p.a. according to age and qualifications plus £450 p.a. London Allowance; superannuation provision.

Applications, giving details of qualifications, experience and the names of two professional referees, should be sent to the Director, National Institute for Medical Research, Mill Hill, London NW7 1AA. 1488(A)



CHEMISTRY
DEPARTMENT

Applications are invited for a Post-doctoral Research Fellowship, supported by the E.E.C. Solar Energy Project D. Fund. With Dr J. M. Kelly and Professor D. C. Pepper the Fellow will study the synthesis and properties of polymer-bound coordination complexes, and assess their potential for solar energy conversion. Previous experience in polymer-, photo-, or coordination compound-chemistry would be helpful.

The salary commences at £3,483 per annum. Funds are available immediately and until June 1979.

Applications should be sent to Dr J. M. Kelly, at the Chemistry Department, Trinity College, Dublin 2, from whom further details may be obtained. 1542(A)

Research Assistant

This Laboratory provides advisory and experimental services to ICI Ltd, for the control of toxic hazards in manufacturing processes and in other fields of growing importance, such as the development of pesticides and food additives. Emphasis is placed on improving and assessing methods for studying the biological effect of foreign compounds and upon understanding mechanisms of toxicity. The provision of toxicological information, prior to product clearance and registration in many countries, is a major activity undertaken by the Laboratory on behalf of the ICI group.

We are seeking to recruit a scientist to assist in the management of the routine short-term tests for the detection of potential carcinogens. The work involves both Ames and cell transformation tests. The person

appointed, male or female, will be responsible for investigating and recommending improvements to protocols where necessary and for the analysis and recording of data. The job will involve contracting work out and the job holder will be responsible for arranging the contract and ensuring that it is of a satisfactory standard.

Requests for application forms, quoting Reference RA/EP/EL, should be addressed to:-

Miss S. C. Carson, Personnel Officer,
Imperial Chemical
Industries Limited,
Central Toxicology
Laboratory,
Alderley Park,
Nr. Macclesfield,
Cheshire.



Overseas candidates should only apply if they have a planned visit to the UK within the next two months.

1537(A)

JUNIOR TECHNICIAN "B"

required to assist in busy immunological laboratory studying pulmonary host responses to infectious agents, inhaled particles and tumours in man and animals. Applicants should hold ONC (MLS), day release granted for HNC studies. Salary on Medical Laboratory Technician Scales. Apply to The Secretary, Cardiothoracic Institute, Fulham Road, London SW3 6HP, giving age, qualifications and experience. 1531(A)

UNIVERSITY OF EAST ANGLIA NORWICH DIRECTOR OF THE CLIMATIC RESEARCH UNIT

Applications are invited from persons with suitable qualifications and experience for the post of Director of the Climatic Research Unit, which is associated with the School of Environmental Sciences. This position will become vacant on the retirement of the present and founding Director of the Unit, Professor H. H. Lamb. The appointment will be at Professorial or Readership level, according to the age and experience of the successful candidate, and will be tenable from October 1, 1978, or as soon as possible thereafter. Salary will be on the Professorial scale £8,106 to £9,587 or on the Reader scale £6,443 to £7,951 (under review).

Applications (one copy only), giving the names of three persons to whom reference may be made, should be lodged with the Establishment Officer, University of East Anglia, Norwich NR4 7TJ (Telephone 0603 56161, ext. 2126) from whom further particulars may be obtained, not later than March 10, 1978. No forms of application are issued. In naming three referees you are particularly requested to give the names of those who can immediately be approached. 1523(A)

DEPARTMENT OF EDUCATION DUBLIN POSTDOCTORAL RESEARCH FELLOWSHIPS

Applications are invited for Research Fellowships in Science (excluding Medical and Social Science) and Engineering tenable in any one of the following institutions in Ireland:—

University College, Dublin;
University College, Cork;
University College, Galway;

An Foras Taluntais
(The Agricultural Institute);

The Institute for Industrial
Research and Standards.

The Fellowships will be tenable for two years with the possibility of extension for a third year.

Applicants should hold a Ph.D. degree or have equivalent research experience or, in the case of engineers or technologists, suitable industrial experience. Candidates should not be over 30 years of age; preferably they should be under 28.

Appointments will be effective not earlier than October 1, 1978. Salary will normally be £3,495 to £3,960 per annum; awards up to £4,617 may be made to engineers and technologists whose industrial experience or other special circumstances warrant it.

Full particulars may be obtained from the Secretary, Department of Education (Headquarters Section), Marlborough Street, Dublin 1. The latest date for receipt by the Department of completed application forms is March 20, 1978. 1507(A)

I.L.R.A.D. DIRECTOR

The position of Director of the International Laboratory for Research on Animal Diseases (I.L.R.A.D.) will become vacant in June, 1978. The Director is the chief officer of the Institution and must have experience and background in disease research and in the administration of a research institution. In addition, the Director must be able to relate to a variety of staff and direct the research programmes of the Institution as well as the various other functions associated with a research programme involving both basic and disease orientated activities.

I.L.R.A.D.'s mandate is to develop effective control methods for trypanosomiasis and theileriosis, two important animal diseases that constrain livestock production in a number of developing countries. It is one of the International Research Centres supported by the Consultative Group on International Agricultural Research. The staff of I.L.R.A.D. is recruited internationally and is engaged in multi-disciplinary research activities leading to the development of immunologic or related control procedures. The I.L.R.A.D. headquarters is in Nairobi, Kenya, and is a modern research complex.

Qualified candidates are requested to submit current C.V.s as well as a synopsis of their research and administrative experiences. Included should be the names and addresses of three referees who can be contacted in support of their candidacy. This information should be submitted to the Chairman, I.L.R.A.D. Search Committee, Rockefeller Foundation, 1133 Avenue of the Americas, New York, N.Y. 10036, U.S.A. Further information can be obtained from the same address. 1559(A)

**FLOUR MILLING AND BAKING
RESEARCH ASSOCIATION
CHEMISTS AND BIOCHEMISTS
STATISTICIAN/MATHEMATICIAN**

The Flour Milling and Baking Research Association carries out a wide range of research and service work for the Milling and Baking Industries and holds a number of research contracts for the Ministry of Agriculture, Fisheries and Food. The laboratories are situated in an attractive semi-rural area on the edge of the Chilterns with ready access to London.

Several positions are available for graduates with strong research backgrounds who would be interested in carrying out basic and applied research on cereal problems.

PROTEIN CHEMIST/BIOCHEMIST

fundamental studies on cereal proteins

ORGANIC CHEMIST/BIOCHEMIST (two posts)

natural product chemistry

CHEMIST/ANALYTICAL CHEMIST

application of modern chemical/physical analytical techniques

BIOCHEMIST

nutritional and toxicological studies

STATISTICIAN/MATHEMATICIAN

statistical and mathematical service for the

Research Association

Applicants should have a Ph.D. degree and further research experience. For the post of Statistician/Mathematician a B.Sc. or equivalent or postgraduate qualification in statistics or mathematics together with several years applied experience is required.

Starting salary will depend on qualifications and experience within the Senior Scientific Officer (c. £4,350 to £5,810 p.a.) or Principal Scientific Officer (c. £5,620 to £7,120 p.a.) scales. There is a pension scheme and assistance with relocation expenses will be given where appropriate.

Further details can be obtained from Professor Brian Spencer, Director-General, Flour Milling and Baking Research Association, Chorleywood, Herts. WD3 5SH. Telephone: 092 78-4111. Applications, with the names and addresses of three referees, should be submitted by March 20. 1404(A)

**UNIVERSITY OF
TORONTO**

Scarborough College

One-year visiting or limited term professorship for 1978/79.

Duties, teaching and research in mathematics. Salary and rank according to qualifications and experience. Send curriculum vitae, and have three letters of reference sent to:

Professor John E. Dove
Chairman
Physical Sciences Division
Scarborough College
University of Toronto
West Hill, Ontario M1C 1A4
Canada 1498(A)

**THE UNIVERSITY
COLLEGE OF WALES
ABERYSTWYTH**

**DEPARTMENT OF BOTANY
AND MICROBIOLOGY**

Applications are invited for the post of Lecturer in Botany. Candidates should have a special interest in an applied aspect of plant ecology e.g. conservation, land use planning or landscaping.

Salary scale: £3,333 to £6,655 p.a. (under review).

Application forms and further particulars available from the Registrar, Old College, King Street, Aberystwyth to whom applications should be sent by March 10, 1978. 1499(A)

**UNIVERSITY OF
EDINBURGH
DEPARTMENT OF
PATHOLOGY**

RESEARCH FELLOW

Applications are invited for the post of Research Fellow to work on a project concerning cells in the allergic response. The successful applicant would probably have experience in biochemistry or pharmacology or immunology. A Ph.D. or equivalent is preferable. The project is supported by the Medical Research Council for a period of three years commencing as soon as possible. The salary will be on Research Range 1A with superannuation.

Applications, including the names of two referees, should be sent as soon as possible to Dr A. B. Kay, Department of Pathology, Medical School, Teviot Place, Edinburgh, from whom further details may also be obtained. Please quote reference 5033. 1527(A)

**MEDICAL
RESEARCH COUNCIL
NATIONAL INSTITUTE
FOR MEDICAL RESEARCH
A POSTDOCTORAL
SCIENTIST**

is required to work on applications of a newly developed technique for separation of cells and macromolecules, using two phase aqueous polymer systems. The candidate will be working in co-operation with engineers and biochemists, and would have a primary responsibility for developments in the biochemical and cell biology side of the project. This is an opportunity for stimulating interdisciplinary research, in which the ability to work and communicate with others is essential.

The appointment will be for up to three years and will be in the present salary range £3,995 to £5,219 p.a. according to age and qualifications plus £450 p.a. London Allowance; superannuation provision.

Applications, giving details of qualifications, experience and the names of two professional referees, should be sent to the Director, National Institute for Medical Research, Mill Hill, London NW7 1AA. 1561(A)

**THE MEDICAL
RESEARCH COUNCIL
DEVELOPMENTAL
NEUROBIOLOGY UNIT
has a vacancy for a
BIOCHEMIST or
CELL BIOLOGIST**

with postdoctoral experience, to join a multidisciplinary research team. The work concerns the isolation and characterisation of cell types from the mammalian brain. Previous experience in membrane biochemistry, protein chemistry or immunology, with an interest in tissue culture, is of advantage. The position (M.R.C. non-clinical staff) is for a year with a possible extension for a period not exceeding three years.

Applicants should send a curriculum vitae giving full details of qualifications and experience and the names of three referees to Dr R. Balazs, M.R.C. Developmental Neurobiology Unit, Institute of Neurology, 33 John's Mews, London WC1N 2NS. 1482(A)

**UNIVERSITY OF IBADAN—
NIGERIA**

Applications are invited for

LECTURESHIP

IN DEPARTMENT OF HUMAN
NUTRITION

Candidates must have a doctorate. This post will specifically be used to encourage studies of energy use and basal metabolic studies in humans. The Department has plans to carry out studies in this field as well as the teaching of undergraduate and post-graduate students. Preference will be given to candidates with expertise in this special field, though candidates with expertise in other fields of human nutrition will also be considered.

Salary scale: N5,460 to N6,984 p.a. (£4,333 to £5,542 p.a. sterling) (£1 sterling=N1.26). There will not be provision of salary supplementation or associated benefits, 25% of salary contract addition (partly taxable) is payable. Family passages; various allowances; superannuation scheme; biennial overseas leave.

Detailed application (2 copies) including a curriculum vitae and naming 3 referees, should be sent by air mail, not later than March 22, 1978, to the Registrar, University of Ibadan, Ibadan, Nigeria.

Applicants resident in U.K. should also send 1 copy to the Inter-University, 90/91 Tottenham Court Road, London W1P 0DT. Further particulars may be obtained from either address. 1549(A)

THE ROYAL SOCIETY

The Royal Society requires

SCIENCE GRADUATE

(up to 30) to assist in its editorial department. General aptitude for scientific editorial work required; some knowledge of mathematics essential and of biological nomenclature desirable.

Progressive salary scale and superannuation scheme. Commencing salary up to £4,200 depending on age and experience.

Applications with names, etc., of two referees to the Executive Secretary, 6 Carlton House Terrace, London SW1Y 5AG, by March 9, marking the envelope 'Personal; W.G.E.'. 1556(A)

**THE
MACAULAY INSTITUTE
FOR SOIL RESEARCH**

DEPARTMENT OF

SOIL FERTILITY

Applications are invited for a

**SCIENTIFIC OFFICER/
HIGHER SCIENTIFIC
OFFICER**

to develop electrochemical studies on nutrient relationships in soils. Candidates should have a First or Upper Second Class Honours Degree, or a Higher Degree or equivalent, in Chemistry, and should be familiar with, and preferably have research experience of, recent developments in electro-analytical techniques and instrumentation. At least two years' relevant postgraduate experience required for appointment as Higher Scientific Officer.

Salary £2,592 to £4,032 (Scientific Officer) or £3,745 to £4,976 (Higher Scientific Officer) per annum including current supplements, according to qualifications and experience. Non-contributory superannuation scheme operative.

Forms of application and further information may be obtained from The Secretary, The Macaulay Institute for Soil Research, Craigiebuckler, Aberdeen AB9 2QJ, to whom they should be returned by 17 March 1978. Quote Ref. 78/3. 1553(A)

**Biochemical Pharmacologist
(£8,000 to £12,000 p.a.)**

A biochemist with considerable theoretical and practical experience in drug metabolism and pharmacokinetics is required to develop and supervise a number of experimental drug studies. Applicants should have at least 3-5 years of postdoctoral experience and would be expected to design and supervise experimental work within the Institute, coordinate external contract studies and be responsible for the final collation and interpretation of results. The International Pharmaceutical Research Institute is located near Munich, has modern research facilities and spoken German is *not* essential. Applicants should hold a passport from an EEC country and would be paid according to age and experience but the salary would be in the range £8,000-£12,000. Applications together with supporting material to Professor C. Kozma, IPHAR, D 8012, Munchen-Ottobrun, Pestalozzistr 25, West Germany. 1557(A)

UNIVERSITY OF EDINBURGH CHAIR OF MATHEMATICS

Applications are invited for the Chair of Mathematics in the University of Edinburgh, left vacant by the recent death of Professor Arthur Erdelyi, F.R.S. The position can be taken up as soon as is convenient to the successful applicant.

As both Pure and Applied Mathematics are represented by the present professors, no particular subject area is prescribed.

Salary within the professorial range, with U.S.S./F.S.S.U.

Further particulars may be obtained from the Secretary to the University, University of Edinburgh, Old College, South Bridge, Edinburgh EH8 9YL with whom applications (12 copies, except for overseas candidates who need submit only one copy), giving the names of three referees, should be lodged by April 15 if possible. Please quote Reference 2/78. 1496(A)

THE QUEEN'S UNIVERSITY OF BELFAST RESEARCH ASSISTANTS DEPARTMENT OF PHYSIOLOGY

Two Research Assistantships are available in the Department of Physiology. The successful applicants will be graduates, preferably holding a first or second class Honours B.Sc. degree. Appointment is tenable initially for one year and may be renewed annually for a further five years.

Salary scale (under review): £2,904 to £4,190, initial placing dependent upon age and qualifications.

Applications giving a full curriculum vitae and the names and addresses of two referees should be addressed to the Personnel Officer, The Queen's University of Belfast BT7 INN, Northern Ireland. Closing date: March 22, 1978. (Please quote Ref. 78/N). 1525(A)

UNIVERSITY OF CAMBRIDGE DEPARTMENT OF PHARMACOLOGY

Applications are invited for an office of:

UNIVERSITY LECTURER IN THE DEPARTMENT OF PHARMACOLOGY

The initial appointment will be for three years from October 1, 1978, with the possibility of re-appointment to the retiring age.

The pensionable stipend for a University Lecturer is on scale £4,607 to £7,087 a year, with initial placing above the minimum where appropriate. There is no grade of Senior Lecturer. A grant is made towards removal expenses.

Candidates should send twelve copies of their application together with the names of not more than three referees to Mr G. R. Anderson, General Board Office, The Old Schools, Cambridge CB2 1TT, from whom further particulars can be obtained, to arrive not later than March 20, 1978. 1417(A)

Product Registration Pharmacist

We are an international pharmaceutical company with European Headquarters in Paris. We are seeking a qualified pharmacist who has an interest in regulatory affairs and who would be responsible, in due course, for the registration of our proprietary products in the U.K., Eire, Scandinavia and english-speaking Africa.

The job can be based in the U.K. or in Paris, but knowledge of French is not essential.

If you:

- are a qualified British pharmacist with at least three years' post-graduate experience, and, preferably, a post-graduate qualification,
- have an interest in the registration of the proprietary products of a successful, expanding international pharmaceutical company,
- are willing to travel extensively between the U.K., Paris and Scandinavia,

then please send a resumé of your career and salary history to:

Mr. R. K. Bryant, Richardson-Merrell Ltd.,
20 Queensmere, Slough, Berkshire SL1 1YY.

1570(A)

UNIVERSITY OF OXFORD

Sir William Dunn
School of Pathology

The University proposes to appoint a
LECTURER

in the Sir William Dunn School of Pathology who will be required to provide teaching in a course for medical students on the principles of cellular pathology and bacteriology and to provide advanced teaching in either cell biology or immunology.

Salary according to age on the scale £3,333 to £7,087 per annum with superannuation.

The post may be held in association with a tutorial fellowship at Wadham College.

Further enquiries concerning both the lectureship and the fellowship should be addressed to Professor H. Harris, F.R.S., Sir William Dunn School of Pathology, South Parks Road, Oxford OX1 3RE, to whom applications (8 copies; one in the case of overseas candidates), together with the names of three referees should be sent by 31 March 1978. 1545(A)

The Polytechnic of North London Physics Department Research Fellow

There is a vacancy for work on low background counting with particular application to measurements of the environmental background of neutrons and to the development of a high efficiency neutron multiplicity detector.

Candidates should have a Ph.D. in experimental physics, preferably with experience in nucleonics, or an equivalent industrial research background.

Fellowships are renewable annually and may be extended up to two or three years. The salary scale is £3,822 to £4,239 (inclusive of London Allowance) with the initial placing depending on previous experience.

Applications giving brief details of experience and qualifications, and requests for further information, should be made to the Head of the Physics Department, The Polytechnic of North London, Holloway, London N7 8DB, as soon as possible. 1489(A)

directeur adjoint de la recherche

160 000 F +

NOUS SOMMES un Centre de Recherche Pharmaceutique Interdisciplinaire - 50 personnes. Nous collaborons avec plusieurs équipes hospitalières ou universitaires tant en France qu'à l'étranger. Nous disposons d'un équipement de pointe et avons élaboré et appliqué avec succès un MODELE NOUVEAU de recherche. Notre Directeur de la Recherche et du Développement crée un poste de DIRECTEUR ADJOINT DE LA RECHERCHE.

SA MISSION sera de coordonner et d'animer une équipe de chercheurs en proposant des méthodologies propres à développer la créativité de chacun, de faire la synthèse des efforts des spécialistes de chaque discipline afin de faire converger les recherches vers la création thérapeutique.

IL FAUT :

- avoir un haut niveau de culture scientifique générale,
- maîtriser les techniques de recherche biologique - biochimie et pharmacologie -
- avoir si possible une expérience post-doctorale de pharmacologie expérimentale,
- parler et lire couramment l'anglais.

LE POSTE convient à un excellent concepteur, de forte ambition scientifique, doué d'enthousiasme et de qualités relationnelles, souhaitant s'épanouir dans l'application de la recherche fondamentale à des fins de réalisation thérapeutique.

Le poste pourra comporter quelques missions à l'étranger.

LIEU DE RESIDENCE région agréable à 200 km de PARIS.

Adresser une lettre manuscrite, ainsi que votre dossier à Madame EDELMAN, qui assurera les premiers contacts dans un souci absolu de discrétion.

CNPG 105, avenue Victor Hugo - 75116 Paris

1543(A)



Geneva Research Centre

seeks

for its Group of Biochemical and Biomedical Research

—an **EXPERIMENTAL PATHOLOGIST** or **TOXICOLOGIST** to develop new areas of research while devoting a portion of his time to the histopathological evaluation of toxicity studies.

—a **PHYSIOLOGIST** with experience in respiratory physiology to participate in an expanding programme in inhalation toxicology.

Knowledge of French would be an asset.

Please address full particulars, with curriculum vitae, photograph and salary requirements to the Personnel Manager, Battelle, 7 route de Drize, 1227 Carouge-Geneva, Switzerland.

1522(A)

THE
ROYAL INSTITUTION
OF GREAT BRITAIN
WOLFSON
PROFESSOR OF
NATURAL
PHILOSOPHY

The Managers of the Royal Institution intend to make an appointment to this newly endowed full-time Chair. Scientists active in physical or biological research, who are also interested in the work of the Royal Institution, are invited to seek further information, from Sir George Porter, Director, The Royal Institution, 21 Albemarle Street, London W1X 4BS.

1554(A)

UNIVERSITY OF
LEICESTER
CHAIR OF
APPLIED PHYSIOLOGY

Applications are invited for a Chair of Applied Physiology within the Department of Physiology, tenable from October 1, 1978 or as soon as possible thereafter. The primary responsibility of the post will be the teaching of medical students and previous experience is essential. There are excellent facilities for research. A medical qualification is desirable but not obligatory. In an appropriate case the Professor would have an Honorary Consultant contract in the N.H.S.

Further particulars may be obtained from the Registrar, The University, Leicester, LE1 7RH, to whom applications (15 copies) should be sent by March 31, 1978 quoting reference NCAP.

1500(A)

VICTORIAN COLLEGE OF PHARMACY LTD.
Melbourne Australia

Position of Dean (Principal) of the College
\$30,786 (Australian)

The Council of the College invites applications for the position of Dean of the College from January 1, 1979, when Dean Nigel C. Manning, C.B.E., retires.

The position is that of the chief executive of the College, and the successful applicant will have enjoyed a broad scientific academic background, preferably in the pharmaceutical sciences, and will have extensive academic and administrative experience.

The occupant will receive a professional salary and an additional allowance of \$4,000 per annum.

Further information on the position may be obtained from the Registrar of the College, with whom applications and the names of three referees should be lodged at 381 Royal Parade, Parkville, Victoria, Australia 3052. The closing date for receipt of applications is April 14, 1978.

Council reserves the right to appoint by invitation.

1146(A)

UNIVERSITY OF WINDSOR
DEPARTMENT OF CHEMISTRY
APPOINTMENT OF
ASSISTANT PROFESSOR

The Department of Chemistry at the University of Windsor expects to have a vacancy for an Assistant Professor, available on July 1, 1978. Applicants should have a Ph.D. preferably in biochemistry and the position will involve teaching in the undergraduate and graduate programs as well as independent research in biochemistry. The Department is well equipped with modern instrumentation and offers a Ph.D. program in biochemistry. Preference will be given to applicants with research experience in metabolism, the biochemistry chemistry of disease, membrane biochemistry or molecular enzymology.

A curriculum vitae with research interests and the names of three referees should be submitted to:

Professor J. E. Drake,
Department of Chemistry,
University of Windsor,
Windsor, Ontario N9B 3P4,
1522(A)

UNIVERSITY OF READING
DEPARTMENT
OF MICROBIOLOGY
RESEARCH FELLOW

required immediately until 14 April 1980 to work on an A.E.R.E. Harwell funded project to identify and quantify microbiological processes occurring in the degradation of domestic and industrial wastes in landfill. Postdoctoral applicants will be given preference but those with equivalent research experience will be considered. The relevant areas of study are microbial ecology and physiology, including fermentation. The work will be closely associated with field and laboratory studies in controlled landfill experiments which are run from Harwell Laboratory by a team of hydrogeologists, chemists and microbiologists for both Department of Environment and Industry.

Starting salary in range £3,333 to £4,190 p.a. (under review) plus U.S.S. superannuation.

Apply for further particulars quoting Ref. M.N.13A, to Assistant Bursar (Personnel), University of Reading, Whiteknights, Reading RG6 2AH,
1568(A)

AQUACULTURE
POSITIONS

University of California, Davis

Faculty positions in a developing aquaculture program. Appointments in the Department of Animal Science, positions housed at Bodega Marine Laboratory. Experience or a strong interest in research and teaching related to the development of aquaculture is essential. **INVERTEBRATE PHYSIOLOGY:** Assistant Professor, 20% teaching and 80% research; supervise a marine program. Ph.D. in an appropriate discipline and expertise in larval physiology, endocrinology or behavioral physiology of marine invertebrates. Teaching at both undergraduate and graduate levels expected. **PATHOLOGY:** Research position, assistant or associate level and lecturer. Ph.D. in fish or invertebrate pathology and interest in research in marine, aquatic-animal pathology and providing diagnosis and treatment for other aquaculture units. Supervision of graduate students expected.

Applicants should submit résumé, letter describing qualifications, publication list, three letters of reference and copies of transcripts to: WALLIS H. CLARK, JR., DIRECTOR OF AQUACULTURE, DEAN'S OFFICE, COLLEGE OF AGRICULTURAL AND ENVIRONMENTAL SCIENCES, UNIVERSITY OF CALIFORNIA, DAVIS, CA 95616 prior to April 15, 1978. Position available July 1, 1978.

The University of California is an Equal Opportunity/Affirmative Action Employer.
1481(A)

MINISTRY OF
OVERSEAS DEVELOPMENT
TROPICAL PRODUCTS
INSTITUTE
BIOCHEMIST/FOOD
SCIENTIST

The above Institute which carries out research to aid developing countries invites applications for a Scientific Officer to work in its New Food Products Section. The work will involve the development of acceptable foods for developing countries, the study of food enzymes in the laboratory, and the analysis of tropical fruits, vegetables and other types of produce. Applicants should have a degree in biochemistry or food science together with broad interests in all aspects of food processing. This post will be a period appointment of two years. Other conditions of service are: Salary £2,614 to £3,992 plus the Government supplements to pay up to a maximum of £522 per annum; 5-day of 41 hours (including lunch breaks); 20 days leave a year; the post is also pensionable.

Application forms from Miss C. A. Hall, Tropical Products Institute, 127 Clerkenwell Road, London EC1R 5DB.
1486(A)

ASSISTANT OR
ASSOCIATE PROFESSOR
ANIMAL PHYSIOLOGY at
CORNELL UNIVERSITY
Division of Biological Sciences

Teach, coordinate senior/graduate course in mammalian physiology, including extensive laboratory section. Candidate should have good background in modern and classical techniques in vertebrate organ physiology. Ph.D., M.D., D.V.M. or equivalent required, with postdoctoral experience desirable. Preference given to applicants with teaching and research expertise in endocrine, skeletal, cardiovascular, respiratory, or renal physiology.

Send curriculum vitae and three letters of recommendation by April 7, 1978 to: Dr R. H. Wasserman, Room 717 Veterinary Research Tower, N.Y.S. College of Veterinary Medicine, Cornell University, Ithaca, N.Y. 14853. An Affirmative Action/Equal Opportunity Employer. Applications from women and minorities are encouraged.
1502(A)

UNIVERSITY OF THE
WEST INDIES—BARBADOS

Applications are invited for the post of **SENIOR LECTURER/LECTURER IN THE DEPARTMENT OF PHYSICS**. Duties to be assumed by September 1, 1978 or as soon as possible thereafter. Salary scales: Senior Lecturer: BDS\$25,827 to 34,017 p.a.; Lecturer: BDS\$19,071 to 29,799 p.a. (£1 sterling=BDS\$3.87). F.S.S.U. Unfurnished accommodation will be let by the University at a rental of 10% of salary. A housing allowance of 20% of salary is payable to staff who make their own housing arrangements. Family passages. Study and Travel Grant.

Detailed applications (three copies) giving full particulars of qualifications and experience, date of birth, marital status and names and addresses of three referees should be sent as soon as possible to the SECRETARY, UNIVERSITY OF THE WEST INDIES, P.O. BOX 64, BRIDGETOWN, BARBADOS. The University will send further particulars for this post to all applicants. These particulars may also be obtained from the Inter-University Council for Higher Education Overseas, 90-91 Tottenham Court Road, London W1P 0DT.

1517(A)

UNIVERSITY OF
SASKATCHEWANSaskatoon, Saskatchewan,
CanadaApplications are invited for the
following positions in the Department
of Crop Science.1. PROFESSIONAL
RESEARCH ASSOCIATEA plant physiologist with training
and experience in the area of water
relations and/or stress physiology.

The successful applicant will be one
of a team of two other scientists and
three technicians who are undertaking
a comprehensive analysis of the role
of plant hormones in drought stress
in sorghum. The study, supported by
the International Development
Research Centre, Ottawa, is designed
to provide cereal scientists working in
the semiarid tropics with a more com-
plete knowledge of the manner in
which plant growth and productivity
are influenced by drought stress. The
project is currently in its third year
and will continue for a minimum of
1.5 years with an anticipated extension
of two further years.

The commencing salary will be
\$16,498 which includes a pension and
other benefits equivalent to 7.9% of
salary. Annual increments in salary
will be in accordance with University
of Saskatchewan increments. Minimum
qualification is a Ph.D. and the
appointee will be expected to join
the group by April 15, 1978. Applica-
tions, which close February 24, should
include curriculum vitae, transcripts
of marks and three letters of reference.

2. PROFESSIONAL
RESEARCH ASSOCIATE

Applications are invited for the
following position as a member of a
multi-disciplinary project on the
biology of wild oats (*Avena fatua*).
A plant physiologist with experience
in the field of seed physiology. Several
years postdoctoral research experience
in the areas of biochemistry and regu-
lation of germination and dormancy is
desirable. The successful applicant will
be expected to direct a full-time tech-
nician and undertake a study of both
the physiological basis of temperature
dependence of seed dormancy and
regulation of endosperm function. The
appointee is expected to interact with
two geneticists, two physiologists and
a herbicide specialist also working
on the project.

The commencing salary will be
\$16,498 which includes a pension and
other benefits. Annual increments will
be in accordance with University of
Saskatchewan increments.

Application which closes on March
15 should include curriculum vitae,
transcripts of marks and three letters
of reference sent to:

Professor G. M. Simpson
Crop Science Department
University of Saskatchewan
Saskatoon, Saskatchewan
Canada S7N 0W0.

1503(A)

SUPERVISOR
RADIOIMMUNOASSAY
FACILITIES

The Department of Obstetrics and
Gynaecology at McGill University has
an immediate opening for a person
experienced in the development, vali-
dation, performance and quality con-
trol of radioimmunoassays for both
steroid and peptide/protein hormones.
Applicants should have a minimum of
a M.Sc. degree (or equivalent), be pre-
pared to work in an academic research
environment and have experience in
the supervision of technicians. Remu-
neration will be commensurate with
professional experience. Please send
curriculum vitae and names and
addresses of three references before
March 15, 1978 to: F. Naftolin, MD,
D.Phil., Professor and Chairman,
Department of Obstetric and Gynaecology,
Women's Pavilion, Royal Victoria
Hospital, McGill University, 687
Pine Avenue West, Montreal H3A
1A1, Canada. An equal opportunities
employer.

1534(A)

UNIVERSITY OF THE
WEST INDIES—JAMAICAApplications are invited for the
following posts:DEPARTMENT OF
MICROBIOLOGY

PROFESSOR. The appointee, will
be expected to give leadership in the
teaching and research activities of the
Department of Microbiology which
prepares students for the M.B., B.S.,
degree of the University of the West
Indies. Experience in Clinical Micro-
biology would be an advantage.

SENIOR LECTURER/LECTURER.
Duties will include routine clinical
Microbiology work for the University
Hospital and instruction in Micro-
biology to students working for the
medical degree of the University of
the West Indies. Preference will be
given to medically qualified applicants.
Duties to be assumed as soon as
possible.

DEPARTMENT OF
BIOCHEMISTRY
(HUMAN NUTRITION)

**LECTURER/ASSISTANT LEC-
TURER.** The appointee is expected
to teach students for the M.Sc.
(Nutrition) degree. He/she should
have a postgraduate degree in bio-
chemistry with special reference to
human nutrition and should have
some knowledge and experience of
nutritional problems in developing
countries. He/she should also have
experience in post-graduate teaching
of human nutrition and should show
evidence of research in nutrition-
related topics. Duties to be assumed
as soon as possible.

Salary scales: Professor-Medical
J\$19,326 to J\$22,578 p.a., Non-
Medical J\$17,166 to J\$21,252 p.a.;
Senior Lecturer-Medical J\$15,339 to
J\$19,326 p.a., Non-Medical J\$12,066
to J\$16,783 p.a.; Lecturer Medical
J\$11,262 to J\$15,015 p.a., Non-
Medical J\$8,913 to J\$13,917 p.a.
Asst. Lecturer Medical J\$9,102 to
J\$9,936 p.a., Non-Medical J\$7,236 to
J\$7,926 p.a.. (£1 sterling equals
J\$2.43). F.S.S.U. Unfurnished accom-
modation will be let by the Univer-
sity at a rental of 10 per cent of
salary, or a housing allowance of
20 per cent of salary is payable.
Study and Travel Grant. Family
passages: baggage allowance. Detailed
applications (three copies) with
curriculum vitae and naming three
referees should be sent as soon as
possible to the Registrar, University
of the West Indies, Mona, Kingston
7, Jamaica. Further particulars of the
post are available from the same
source or from the Inter-University
Council for Higher Education Over-
seas, 90-91 Tottenham Court Road,
London W1P 0DT. 1518(A)

UNIVERSITY COLLEGE
HOSPITAL MEDICAL
SCHOOLDEPARTMENT OF CLINICAL
PHARMACOLOGYLaboratory of Toxicology and
PharmacokineticsRESEARCH WORKER IN
PHARMACOKINETICS

A position, probably at postdoctoral
level, is vacant in a small active team
working on problems of drug metabo-
lism and toxicity. The applicant
should have experience with H.P.L.C.
or G.C. techniques, and be interested
in research relating plasma levels of
drugs and metabolites to drug action.

Starting salary in the range of
£3,500 to £4,200, depending on qual-
ifications and experience. The appoint-
ment is for three years.

Applicants should apply in writing,
enclosing a curriculum vitae and
names of two referees, to:

Dr A. McLean,
University College Hospital
Medical School,
University Street,
London WC1

1539(A)

CSIRO
AUSTRALIAPostdoctoral
Research Fellow
Molecular and Cellular Biology Unit
North Ryde, N.S.W.

CSIRO has a broad charter for research into primary and
secondary industry areas. The Organization has approximately
7,000 employees—2,300 of whom are research and pro-
fessional scientists—located in Divisions and Sections
throughout Australia.

General: The newly established Unit forms, with part of the
Division of Animal Production, the CSIRO Genetics Research
Laboratories. Its objective is the study of basic mechanisms of
Molecular Genetics and Cellular Biology and their applica-
tions to problems of human welfare, animal production and
industry. The Unit has ten research scientists with some 21
support staff and 15 site service staff. The Unit is well equipped
for its present activities in Molecular Biology and has facilities
for small animal research. It has close working relations with
other Divisions of Applied Organic Chemistry, Food Research,
Protein Chemistry and Animal Production; the Universities of
New South Wales, Macquarie and the Australian National
University, Canberra. The laboratory is situated in a pleasant
suburban location close by other CSIRO establishments and
Macquarie University.

Duties: To work on the chemistry of mutagenesis, or DNA
breakage and repair, or the isolation, characterization and
sequencing of DNA/RNA associated with the differentiation
and evolution projects of the Unit. The Fellow would be
expected to collaborate with one of the existing groups in the
Unit which are studying DNA breakage and repair, cellular
differentiation, molecular evolution of influenza virus and
antigenic diversity. Experience with the enzymology of DNA
replication or degradation, of recombinant DNA technology
or of nucleic acid sequencing, or in some similar area would
be an advantage.

Salary: Research Scientist or Senior Research Scientist:
A\$14,506 to A\$21,134 p.a., (UK £8,703 to UK £12,680),
(US \$16,536 to US \$24,092).

Tenure: The appointment will be for a fixed term of 3-5 years.

Applications (in duplicate), stating *FULL* personal and
professional details, the names and addresses of at least two
professional referees, and quoting reference number 690/15
should reach:—The Personnel Officer, Australian Scientific
Liaison Office, Canberra House, Maltravers Street, LONDON
WC2R 3EH by 22nd March 1978.

Applications in U.S.A. and Canada should be sent to:—
The Counsellor (Scientific), Embassy of Australia, 1601
Massachusetts Avenue, N.W. WASHINGTON D.C. 20036
U.S.A. 1512(A)

nature

Nature is considering employing a physical scientist
to assist Dr Stuart Sharrock, the Physical Sciences
Editor. Such a person would have recently received a
Ph.D. and would be hoping to develop a much broader
appreciation of science. He or she would be expected to
help Dr Sharrock in the assessment of manuscripts,
work with other members of the *Nature* staff in com-
piling the various sections of the journal, establish
contacts in the scientific community and, on occasions,
write short pieces on recent scientific developments.

Write to Editor, *Nature*, 4 Little Essex Street, London
WC2 enclosing a c.v. and any other material you
consider relevant. 1430(A)

CHEMICAL ENGINEER

Challenging Experimental and Design Work

This post, based at Culham, Oxon, covers a wide spectrum of project work in relation to the processing of plant and animal produce. The main emphasis is on dehydration studies undertaken by an interdisciplinary team.

Developed processes are field tested overseas and short visits will be made to commission equipment and supervise field trials.

Candidates normally under 30 must have a degree or equivalent in Chemical Engineering. Some relevant post-graduate experience is essential.

Starting salary, as Higher Scientific Officer, will be within the range £3740—£4975.

For further details and application form (to be returned by 17 March 1978) please write to Miss C A Hall, Tropical Products Institute, 127 Clerkenwell Road, London, EC1R 5DB. Please quote ref: SA/78/JD. 1484(A)

Ministry of Overseas Development Tropical Products Institute

UNIVERSITY OF BATH LECTURERS IN PHARMACOLOGY

Applications are invited for two posts in the School of Pharmacy and Pharmacology. One of the posts is temporary for a period of two years.

Further particulars are available from the Personnel Officer, University of Bath BA2 7AY, quoting reference number 78/38N. Closing date for applications March 13, 1978. 1487(A)

UNIVERSITY OF THE WITWATERSRAND Johannesburg DEPARTMENT OF GEOLOGY CHAIR OF MINERAL GEOCHEMISTRY

Applications are invited for appointment to the above teaching and research position. Applicants should have an extensive research interest in the application of analytical or experimental mineral chemistry and/or thermodynamics to petrogenetic problems. The successful candidate will be responsible for the co-ordination and advancement of teaching-courses in geochemistry in the department; and, in addition to pursuing his own research projects, will be encouraged to participate in co-operative research programmes in a wide range of interdisciplinary Earth Science projects. A modern ARL SEMQ electron microscope has recently been installed, and a supporting post of electron microprobe operator has been advertised.

The starting salary will be determined according to qualifications and experience in the range R10,800 to R13,800 per annum (plus a 10% pensionable allowance).

The University's policy is not to discriminate in the appointment of staff or the selection of students on the grounds of sex, race, religion or colour. Further particulars relating to this practice are included in an information sheet obtainable from the London Representative, University of the Witwatersrand, 278 High Holborn, London WC1V 7HE, or from the Registrar, University of the Witwatersrand, Jan Smuts Avenue, Johannesburg, South Africa, 2001, with whom applications should be lodged not later than 17 March 1978. 1563(A)

UNIVERSITY OF OXFORD RESEARCH ASSISTANT

required for three years to work on aspects of muscle metabolism after severe injury. Experience in using an amino acid analyser desirable.

Pay and conditions of service on Whitley Council Biochemist Scale, from £3,560 p.a.

Applications and further information from Dr R. Smith, Tayfield Orthopaedic Centre, Headington, Oxford, or Dr D. H. Williamson, Metabolic Research Laboratory, Nuffield Department of Clinical Medicine, Radcliffe Infirmary, Oxford. 1558(A)

UNIVERSITY OF SURREY DEPARTMENT OF BIOCHEMISTRY EXPERIMENTAL OFFICER

Applications are invited for the post of Experimental Officer in the Department of Biochemistry, to work with the Head of Department, Professor D. V. Parke. The person appointed (male or female) will assist in the development of research services in the Department and carry out research work in the following areas:

metabolism and pharmacokinetics of drugs and environmental chemicals, mechanisms of drug and chemical toxicity, mechanisms of carcinogenesis, regulation of glycoprotein synthesis.

Applicants must be graduates, preferably with a higher degree in biochemistry or pharmacology and experience in biochemical and pharmacological techniques. Industrial experience would be considered an advantage.

Salary will be within the Experimental Officer range £3,975 to £5,015 per annum depending on experience and qualifications.

Applications in the form of a curriculum vitae, giving full details of age, experience and qualifications (and the names and addresses of two referees), should be sent to the Assistant Secretary (Personnel), University of Surrey, Guildford, Surrey GU2 5XH, by: March 16, 1978, and from whom further particulars of the post can be obtained: Telephone Guildford 71281, Ext. 452. 1506(A)

UNIVERSITY OF READING DEPARTMENT OF AGRICULTURAL BOTANY RESEARCH DEMONSTRATOR

required as soon as possible. Duties will include up to 12 hours per week demonstrating in practical classes related to the degree in Agricultural Botany. Candidates should have a good honours degree in Agricultural Botany, Botany or Biology, and will be expected to register for a higher degree, M.Phil. or Ph.D. Three-year appointment.

Salary in scale £2,550 to £2,750 p.a.

Applications, together with the names of two referees, should be made to Professor Watkin Williams, Department of Agricultural Botany, University of Reading, Whiteknights, Reading RG6 2AS, from whom further particulars may be obtained. Closing date: 14 March 1978. (Ref. T.N.14A.) 1569(A)

MEMORIAL UNIVERSITY OF NEWFOUNDLAND FACULTY POSITION Ph.D. FOOD SCIENTIST, TEACHING AND RESEARCH

The Department of Biochemistry, Memorial University of Newfoundland is seeking an individual for teaching and research with a new undergraduate and graduate program of Food and Nutrition. Applicants should have a demonstrated ability for research in Food Science. An interest in fish and other marine sources of food is highly desirable. Date of availability on or before September, 1978. Résumé should include biography, transcripts and names of references. Respond to Dr Norman F. Haard, Department of Biochemistry, Memorial University of Newfoundland, St John's, Newfoundland, Canada A1B 3X9. 1541(A)

RESEARCH TECHNICIAN

for biochemical laboratory working on biosynthesis and intracellular transport of proteins. Applicants should have a good technical background in general biochemistry.

Salary on Whitley Technicians' scale.

Apply in writing to Mr C. G. Keast, University College Hospital Medical School, University Street, London WC1, including a curriculum vitae and names of two referees. 1551(A)

UNIVERSITY OF READING DEPARTMENT OF GEOLOGY RESEARCH DEMONSTRATOR IN GEOLOGY

required to assist with undergraduate laboratory and field classes; should have special interests in the petrology and geochemistry of crystalline rocks. Research for a higher degree will be on mineral deposition from high temperature volcanic fluids. Post available now but persons expected to graduate in summer 1978 eligible to apply. Appointment for one year in first instance, renewable. Salary in scale £2,550 to £2,750 p.a.

Further particulars from Professor P. Allen, F.R.S., Department of Geology, University of Reading, Whiteknights, Reading RG6 2AB. Closing date for applications March 13, 1978. (Ref. T.N.13A.) 1504(A)

RESEARCH ASSOCIATE POSITIONS

at the Lady Davis Institute, Montreal, will be available July, 1978 for molecular biologists to join group investigating legume gene activity during symbiotic nitrogen fixation.

Salary \$11,000 to \$13,000 per annum.

Applicants with expertise in mRNA isolation, cDNA synthesis and characterisation or restriction endonuclease mapping should contact Dr H. M. Schulman, John Innes Institute, Norwich, England. 1477(A)

EUROPEAN TISSUE CULTURE SOCIETY— GLASGOW

July 3–5, 1978

Main topics—differentiation in Friend cells and teratocarcinoma, somatic cell genetics, characterisation of tumour cells *in vitro*, plus workshops and poster sessions on a wide range of topics related to tissue culture.

Enquiries to Doctor Ian Freshney, Beatson Institute for Cancer Research, Gartcube Estate, Bearsden Road, Glasgow G61. 1510(C)

UNIVERSITY OF CALGARY DEPARTMENT OF PHYSICS

RESEARCH ASSISTANT required to carry out observational and experimental work related to the LOWER ATMOSPHERE. The successful candidate should either have experience or an interest in one of the following: aerosol science, atmospheric visibility, urban pollution, lidar probing or atmospheric optics; and must be able to handle associated equipment both in the laboratory and in the field. This position provides an excellent opportunity to gain experience in environmental physics in association with a relatively small and friendly group of scientists, technicians, and graduate students. B.Sc. and Ph.D. graduates may apply. Aptitude for experimental work and relevant experience considered as important as basic qualification.

Salary range, \$9,600 to \$14,400.

Apply to Professor A. W. Harrison, Department of Physics, University of Calgary, Alberta, Canada, including a brief résumé of background and experience.

UNIVERSITY OF SASKATCHEWAN COLLEGE OF DENTISTRY SASKATOON, CANADA ORAL BIOLOGY

Applications are invited for two full-time positions in the Department of Oral Biology. Depending on qualifications, applicants may be considered for Chairmanship of the Department.

ORAL PATHOLOGY: The applicant should have a dental degree and graduate training in Pathology—Oral and/or General. Responsibilities will include teaching at the undergraduate level, research and managing a diagnostic histopathology service.

ORAL BIOCHEMISTRY/MICROBIOLOGY: The applicant should have a dental degree and graduate training preferably beyond the Masters level. Responsibilities will include teaching at the undergraduate level and research in biochemistry/microbiology.

Rank and salary will be determined according to qualifications and experience.

Letters of application and curriculum vitae should be sent to: Dr E. R. Ambrose, Dean, College of Dentistry, University of Saskatchewan, Saskatoon, Saskatchewan, Canada S7N 0W0 1442(A)

UNIVERSITY OF ESSEX DEPARTMENT OF BIOLOGY

The following posts, funded by a grant from the Medical Research Council to Dr R. W. Davies, are available immediately and tenable for three years.

Both are for work on the structural basis of site-specific recombination of bacteriophage lambda, using modern nucleic acid sequencing methods.

POSTDOCTORAL RESEARCH FELLOW (Ref. AG/19)

Starting salary £3,333 p.a. on Range 1A of Auxilliary (Research) Staff scale. Applicants having experience in nucleic acid biochemistry or molecular biology preferred.

RESEARCH TECHNICIAN (Ref. P36)

Starting salary £3,186 p.a. of Technician Grade 5. Applicants should have extensive research experience.

Two copies of applications (quoting the appropriate reference) including a curriculum vitae and the names and addresses of two referees, should reach the Registrar, University of Essex, Wivenhoe Park, Colchester CO4 3SQ, from whom further particulars may be obtained by 16 March. 1567(A)

UNIVERSITY OF EDINBURGH DEPARTMENT OF GEOLOGY A RESEARCH ASSOCIATE

is required to assist in operating a Microscan 5 electron probe analytical unit, jointly supported by the Natural Environment Research Council and the University of Edinburgh. The contract will be for a period of 28 months in the first instance to start as soon as possible.

Salary will be on research range 1B or 1A, according to age and experience. The appointee will be expected to pursue his/her own and collaborative research topics; in addition he/she will be responsible for user instruction, and the coordination and supervision of an analytical service to other workers. The position will carry superannuation under normal university schemes.

Applications (2 copies) should be sent to:

Professor M. J. O'Hara
Grant Institute of Geology
West Mains Road
Edinburgh EH9 3JW
by March 15, 1978, from whom further particulars can be obtained.
Please quote: Reference 5004. 1546(A)

THE MIDDLESEX HOSPITAL MEDICAL SCHOOL (University of London) BIOPHYSICAL ENDOCRINOLOGY UNIT, DEPARTMENT OF PHYSICS AS APPLIED TO MEDICINE

Applications are invited for a Research Assistant 1A; starting salary, depending on age and qualifications, of £3,783 to £4,212 including £450 per annum London Allowance.

Suitable for a postdoctorate, this post will be available for two years, funded by a Medical Research Council Programme Grant. The project will involve the in vitro assay of pressor substances including the preparation and use of dispersed muscle cells. Reproduction and investigation of other biological cells may be involved.

Applications, together with the names of two referees should be sent to the Secretary, Department of Physics as Applied to Medicine, Hindey Building, Cleveland Street, London W1P 6DB. 1462(A)

UNIVERSITY OF NAIROBI KENYA

Applications are invited for the following posts:

DEPARTMENT OF CROP SCIENCE SENIOR LECTURER

Applicants should have a basic degree in Agriculture or Horticulture plus a Ph.D. in Agronomy, Agricultural Botany, Crop Physiology, Plant Breeding or Crop Protection. Experience in teaching and research is essential. The applicant should participate in teaching undergraduate and postgraduate courses and in the department's research activities.

DEPARTMENTS OF SOIL SCIENCE AND BOTANY (MIRCEN PROJECT)

SENIOR LECTURER/ LECTURER (Microbiologist)

Applicants should possess a Ph.D. in either Soil Science or Microbiology with considerable relevant university teaching and research experience in legume bacteriology. Expertise on nitrogen fixation entailing rhizobia-legume symbiosis is a desirable advantage. The appointee will be expected to teach undergraduate and postgraduate courses, conduct research on nitrogen fixation in collaboration with the University of Nairobi U.N.E.P., U.N.E.S.C.O., I.C.R.O. (MIRCEN) project. Appointment will be on contract for two years.

Salary scales: Professor K£4,632 to K£5,562 p.a.; Senior Lecturer K£2,988 to K£3,984 p.a.; Lecturer K£2,016 to K£3,312 p.a. (K£1=£1.29 sterling.) The British Government may supplement salaries in range £3,354 to £4,674 p.a. (sterling) for married appointees and £2,184 to £3,384 p.a. (sterling) for single appointees (reviewed annually and normally free of all tax) and provide children's education allowances and holiday visit passages. Terms of service include family passages: superannuation and medical aid scheme; subsidised housing and various allowances.

Detailed applications (2 copies) with curriculum vitae and naming 3 referees to be sent to Registrar, University of Nairobi, P.O. Box 30197, Nairobi, Kenya, by March 15, 1978.

Applicants resident in U.K. should send one copy to Inter-University Council for Higher Education Overseas, 90/91 Tottenham Court Road, London W1P 0DT. Further details may be obtained from either address. 1548(A)

MATHEMATICS GRADUATE STUDY

Graduate Assistantships paying up to \$6,000 per year plus tuition are available in the Mathematics Department, Clarkson College, Potsdam, N.Y. 13676.

Clarkson offers a well-rounded curriculum with emphasis in applied mathematics, particularly in the following fields: Matrix Methods in Systems Science, Mathematical Physics, Computer Science, Statistical Methods in Oil and Mineral Resource Prediction, Nonlinear Waves, Numerical Analysis, and Functional Analysis. For additional information write to Dean of the Graduate School, Clarkson College, Potsdam, NY 13676.

Clarkson College is an equal opportunity/affirmative action employer. X1533(A)

UNIVERSITY OF WARWICK RESEARCH FELLOW

Applications are invited for a S.R.C. postdoctoral research fellowship for two years in the Department of Physics, to work on the diffusion of hydrogen and deuterium in transition metals using pulsed field gradient nuclear magnetic resonance. Experience in N.M.R. is desirable but not essential. Initial salary will be at not higher than point 3 (£3,761 p.a.) on the Range 1A scale £3,333 to £5,627 p.a. (under review). Further particulars and application forms from the Academic Registrar, University of Warwick, Coventry CV4 7AL quoting Ref. No: 28/R/78. 1513(A)

AGRICULTURAL RESEARCH COUNCIL

FOOD RESEARCH INSTITUTE SENIOR PRINCIPAL SCIENTIFIC OFFICER

HEAD OF THE CHEMISTRY DIVISION

Applicants should have a broad qualification in Chemistry and distinction in a relevant research area with a biological or biochemical background since a significant element of biochemical research will be included in this Division.

Physical and biophysical interests will be the responsibility of a parallel division to be created in the near future. Experience related to the interests of the Food Industry will be of especial value. The total staff of the Division will be approximately 40, including at least six Principal Scientific Officers and support staff divided into groups broadly concerned with Lipid, Carbohydrate, Protein, Natural Product and Biochemistry in relation to food.

The Institute is housed in virtually new laboratories in Norwich and takes a major role in the ARC research effort on food in the UK. Further details are available in a document which outlines some of the proposed developments.

The salary range for this appointment is £8,858 to £10,006 per annum, including Phase II pay supplement, and there is a non-contributory superannuation scheme.



Application forms (and further particulars) are available from the Secretary, ARC Food Research Institute, Colney Lane, Norwich NR4 7UA, to whom they should be returned by 3rd April 1978. 1519(A)

POSTDOCTORAL IMMUNOBIOLOGIST

required to join research team concerned with the control of IgE antibody responses.

Particular emphasis will be directed towards assessing the effects of varying antigen structure on suppressor cell activity. Applicants should have considerable experience with cellular immunological techniques.

Application forms from: Mrs Nancy Morgans, Personnel Manager, Miles Laboratories Ltd, Stoke Court, Stoke Poges SL2 4LY. Tel: Farnham Common 2151. 1524(A)

NOTE CHANGE IN PREVIOUSLY ANNOUNCED POSITION FOR VERT. PALEONTOLOGIST, UNIVERSITY OF KANSAS. The 9 mo. 1-time position in Sys. & Ecol. will be expanded to 12 mo. 1-time. The Mus. of Nat. Hist. position remains 12 mo. 1-time and the combined salaries will be 16,000-18,000. The closing date for applications will be extended to March 30, 1978. Apply: Larry Martin, 602 Dyche, Univ. of Kansas, Lawrence, KS 66045. Equal Opportunity Employer. 1535(A)

UNIVERSITY COLLEGE CORK DEPARTMENT OF ELECTRICAL ENGINEERING FULL-TIME APPOINTMENT

The Governing Body invites applications for a full-time post as Assistant Lecturer/College Lecturer in the Department of Electrical Engineering. The appointment will be made at one of the levels mentioned according to the qualifications and experience of the successful candidate.

The salary scales are:—
College Lecturer £5,115 to £6,121 to Bar to £6,141 to £7,340 p.a.
Assistant Lecturer £4,403 to £4,805 p.a.

Application form and further details of the post may be obtained from the undersigned.

Latest date for receipt of applications is Thursday, March 16, 1978.

M. F. Kelleher
Secretary

UNIVERSITY COLLEGE CORK DEPARTMENT OF STATISTICS FULL-TIME APPOINTMENT

The Governing Body invites applications for a full-time post as Assistant Lecturer/College Lecturer in the Department of Statistics. The appointment will be made at one of the levels mentioned according to the qualifications and experience of the successful candidate.

The salary scales are:—
College Lecturer £5,115 to £6,121 to Bar to £6,141 to £7,340 p.a.
Assistant Lecturer £4,403 to £4,805 p.a.

Application form and further details of the post may be obtained from the undersigned.

Latest date for receipt of applications is Thursday, March 16, 1978.

M. F. Kelleher
Secretary
1497(A)

MEDICAL RESEARCH COUNCIL LABORATORY ANIMALS CENTRE

Carshalton, Surrey

Applications are invited for the post of Senior Research Officer in the Director's Department.

The successful candidate will be expected to assist the Director with:

- (i) personal assistance with his administrative duties
- (ii) a research programme designed to evaluate specific pathogen free cats.

It is hoped that he/she will be a mature person with an interest in scientific administration. Applicants should have a degree in biological science, together with a Ph.D., and previous experience with small animals would be an advantage although not essential. The post offers excellent conditions of service. Salary in the range £3,999 to £5,199 according to age, qualifications and experience, plus £354 London Weighting, plus £312 Pay Supplement, plus Variable Pay Supplement. Contributory Superannuation Scheme.

Applications, together with a curriculum vitae and the names of two referees should be sent to the Director, Medical Research Council Laboratory Animals Centre, Woodmansterne Road, Carshalton, Surrey SM5 4EF.

1377(A)

MRC

Medical Research Council

THE MIDDLESEX HOSPITAL MEDICAL SCHOOL (University of London) DEPARTMENT OF MEDICINE RESEARCH ASSISTANT

Applications are invited for the post of Research Assistant to work on the development of Homologous Assays for Human Parathyroid Hormone. Candidates should preferably have a Ph.D. and experience with immuno-assays is desirable. The post is available for three years.

Starting salary, according to age and qualifications, up to £3,996 per annum inclusive of £450 per annum London Allowance.

Applications, including curriculum vitae and the names and addresses of two referees, should be submitted to Dr J. L. H. O'Riordan, Department of Medicine, Sir Jules Thorn Institute of Clinical Science, The Middlesex Hospital Medical School, London W1P 7PN. 1529(A)

UNIVERSITY OF OTAGO Dunedin, New Zealand DIRECTOR, ANIMAL BREEDING STATION

Applications are invited from graduates in either veterinary, biological or agricultural science with training and experience or interest in small animal husbandry to take charge of the University Animal Breeding Station and Laboratory Animal Sciences Unit of the Medical School and be consultant on housing and care of laboratory animals within the University system. The Director will be encouraged to pursue his own research interests.

Appointment carries salary and academic status of Lecturer or Senior Lecturer.

Salary: Lecturer NZ\$10,585 to \$12,969; Senior Lecturer NZ\$13,424 to \$16,546 with a bar at \$15,411 per annum.

Further particulars are available from the Association of Commonwealth Universities (Appts), 36 Gordon Square, London WC1H 0PF, or from the Registrar of the University.

Applications close on May 15, 1978. 1540(A)

UNIVERSITY OF STIRLING DEPARTMENT OF BIOLOGY UNIT OF AQUATIC PATHOBIOLOGY

Applications are invited for a post as Lecturer in Aquaculture from June 1, 1978. The successful candidate will be required to teach in the M.Sc. and diploma courses run by the Unit and to undertake research in an appropriate field. Preference will be given to applicants who are also qualified to teach in undergraduate courses in Biology. Salary scale £3,333 to £6,655 plus F.S.S.U./U.S.S. (currently under review).

Applications, naming two referees, which should be received by March 15, 1978 by The Secretary (N), University of Stirling, Stirling FK9 4LA, from whom further information is available. 1526(A)

UNIVERSITY OF QUEENSLAND Australia

RESEARCH FELLOW Platypus Ecology Project

This project is funded by the Australian National Parks and Wildlife Service. It aims to provide information on various aspects of the ecology of the platypus (*Ornithorhynchus anatinus*), such as distribution, population dynamics, dietary requirements, environmental parameters and reproductive ecology. The successful applicant should hold (or expect to be awarded in the near future) a Ph.D. or equivalent and have experience in the conduct of field investigations. Consideration may be given to the appointment of an otherwise well qualified applicant, who does not hold a Ph.D. on the Senior Research Assistant scale. The appointment will be for one year in the first instance and the appointee should be prepared to take up his duties as soon as possible.

Closing date for application is 31 March 1978, and further information may be obtained from the Chief Investigator (F. N. Carrick), Reproductive Biology Group, Department of Veterinary Anatomy, University of Queensland, St. Lucia, Brisbane 4067, Australia. (Quote Reference No. 06578.)

Salary: \$A14,851 to \$A23,299 per annum. 1564(A)

STUDENTSHIPS

GLASSHOUSE CROPS RESEARCH INSTITUTE invites applications for an AGRICULTURAL RESEARCH COUNCIL STUDENTSHIP

tenable at the Institute for three years for studies with *Verticillium fungicola* the casual organism of dry bubble disease in cultivated mushroom.

Minimum qualification first or upper second class honours degree in relevant subject.

Application forms and further particulars available from Secretary, G.C.R.I., Worthing Road, Rustington, Littlehampton, Sussex. Closing date for applications March 20. 1509(F)

UNIVERSITY OF BRISTOL LONG ASHTON RESEARCH STATION AGRICULTURAL RESEARCH COUNCIL STUDENTSHIP

A studentship will be offered to an honours graduate (first or upper second class) in appropriate science subjects for research on one of the following topics:—

1. The effects of leaf diseases on yield potential in cereals.
2. Cytogenetic basis of fungicidal action and resistance mechanisms.
3. The biosynthesis of antifungal amine conjugates in barley.

Candidates should normally be under 27 years of age on October 1, 1978 and graduates of a British Commonwealth University, being British subjects normally resident in the United Kingdom. The successful applicant will be expected to register for a higher degree in the University of Bristol.

Application forms and further particulars can be obtained from The Secretary, Long Ashton Research Station, Long Ashton, Bristol BS18 9AF. The closing date for receipt of completed application forms is March 17, 1978. 1520(F)

SCOTTISH HORTICULTURAL RESEARCH INSTITUTE Invergowrie, Dundee DD2 5DA Agricultural Research Council

POSTGRADUATE STUDENTSHIP

Applications are invited for a Studentship tenable for 3 years from October 1978 in the Virology Section of the Institute. Research will centre on the properties of viruses infecting carrot, parsnip and other umbelliferous plants, and the way in which the viruses are transmitted by aphids. A background of plant pathology, cell biology or entomology is desirable.

The minimum qualification is an Upper Second Class Honours degree. Students graduating in 1978 are eligible.

Application forms and further particulars are available from the Secretary of the Institute. The closing date for applications is March 31, 1978. 1480(F)

WELSH PLANT BREEDING STATION

University College of Wales,
Aberystwyth
A.R.C. RESEARCH
STUDENTSHIP

Applications are invited for an A.R.C. Research Studentship tenable for three years from September October 1978 on one of the following research topics:

1. Sources of resistance to diterous stem borers in Italia and perennial ryegrass. (Supervisor: Mr J. A. Brook)
2. The basis of durable resistance to cereal mildew. (Supervisor: Dr T. L. Carver)
3. Sources and mechanisms of resistance to yellow rust in barley. (Supervisor: Dr B. C. Clifford)

The candidate, who should possess or expect to gain a good Honours degree in a relevant discipline, will be registered with the University of Wales for a higher degree.

Applications, including the names of two referees, should be sent before March 6, 1978 to The Secretary, Welsh Plant Breeding Station, Plas Gogerddan, Aberystwyth, Dyfed SY23 3EB from whom further particulars can be obtained. 1479(F)

THE WEST OF SCOTLAND AGRICULTURAL COLLEGE Crichton Royal Farm, Dumfries

RESEARCH STUDENTSHIP

Applications are invited for Research Studentship at the above experimental husbandry farm, to study herbage production and its utilisation by dairy cattle. The current value of the award, which will be tenable for up to three years, is £1,478 p.a. plus fees and approved travel expenses.

Applicants should have a first or second class honours degree in agriculture or agricultural science and the successful applicant will be required to register for a higher degree.

Further details may be obtained from the Secretary, The West of Scotland Agricultural College, Auchincruive Ayre KA6 5HW, with whom applications should be lodged by March 17, 1978. 1457(F)

UNIVERSITY OF ABERDEEN FACULTY OF MEDICINE RESEARCH STUDENTSHIPS

Applications are invited from recent first or upper second class honours graduates in a biological science subject, and from those who expect so to graduate in 1978, for research studentships established by the Faculty of Medicine from its Medical Endowment Funds. Successful applicants will study for the degree of Ph.D. on an approved subject in one of the Departments of Biochemistry, Developmental Biology, Pathology, Pharmacology or Physiology, commencing in October 1978.

The value of the studentship is £1,475 p.a. (for students living away from home) with payment of tuition fees and other allowances.

Application forms and further particulars of the projects may be obtained from D. K. Yule, University Office, Regent Walk, Aberdeen AB9 1FX, to whom applications should be submitted by March 17, 1978. 1468(F)

STUDENTSHIPS—continued

AWARD OF STUDENTSHIP
from October 1978

Applications are invited from graduates or persons with appropriate qualifications for the following postgraduate studentship, tenable from October 1978 at the stipend shown with remission of composition and student society fees:

Associated Lead Manufacturers Limited Studentship

for research in Chemistry, Physics or Metallurgy; £1,475 per annum; normally for three years. Further particulars and application forms may be obtained from the office of the Registrar to whom completed application form should be submitted by 2 June 1978. 1566(F)

EAST MALLING
RESEARCH STATION
AGRICULTURAL
RESEARCH COUNCIL
STUDENTSHIP

Postgraduate studentship tenable from October 1, 1978 for three years or research leading to higher degree in either:

(a) The role of phenolic glycosides in plant growth

or
(b) Earthworm populations in orchards as affected by soil management systems.

First or Upper-second Honours degree essential.

Further details and application form from Assistant to the Secretary, East Malling Research Station, Maidstone, Kent ME19 6BJ. 1528(F)

WYE COLLEGE
(University of London)
DEPARTMENT
OF HOP RESEARCH
AGRICULTURAL
RESEARCH COUNCIL
STUDENTSHIP

Applications are invited for a studentship tenable for three years, commencing October 1978, in the pathology Section of the Department. The research project will be on the application of mathematical methods to the dynamics of epidemic development. It will include experimental and analytical work on hop mildew diseases and the evaluation of quantitative methods in general.

Applicants should hold, or expect to hold, a first or upper second class honours degree in either biological science or mathematics. They need to combine aptitude in mathematical, statistical and computer techniques with a keen interest in their application to biological systems.

Application forms and further particulars may be obtained from the registrar, Wye College, Wye, Ashford, Kent TN25 5AH. 1565(F)

DUBLIN INSTITUTE FOR
ADVANCED STUDIES
SCHOOL OF
THEORETICAL PHYSICS

Applications are invited for research Scholarships tenable in the school during the academic year 1978-79. A limited number of stipends will be available. These awards are normally made at postdoctoral level. For further particulars and Forms of Application, candidates should apply immediately to The Registrar, Dublin Institute for Advanced Studies, 10 Burlington Road, Dublin 4, Ireland. Completed applications should reach the Registrar not later than April 3, 1978. 1544(H)

FELLOWSHIPS

UNIVERSITY COLLEGE OF
NORTH WALES, BANGOR
SCHOOL OF PHYSICAL AND
MOLECULAR SCIENCES
POSTDOCTORAL
FELLOWSHIP IN
ORGANIC CHEMISTRY

Applications are invited for a Postdoctoral Fellowship funded by the I.C.T. Joint Research Scheme for work on the application of sulphur compounds.

The appointment, to the Research and Analogous Scale IA is within the range £3,761 to £4,190 per annum, and will be for a two-year period, commencing June 1, 1978, or such later date as may be arranged.

Applications, giving details of qualifications, research experience and the names and addresses of two referees, should be sent, as soon as possible, to the Assistant Registrar (Personnel), University College of North Wales, Bangor, Gwynedd LL57 2DG. 1501(E)

UNIVERSITY OF
WARWICK
RESEARCH FELLOWSHIP
IN VIROLOGY

Applications are invited for a Postdoctoral Fellowship in the Tumour Virus Research Group in the Department of Biological Sciences. The successful applicant will work in a small group studying the biochemistry of C type RNA tumour viruses, with particular reference to their ability to transform cells. Experience of nucleic acid technology, while not essential, would be an advantage.

Starting salary will be between points 1 and 6 on the Research Range IA scale: £3,333 to (£4,403) to £5,627 p.a. (under review). The Fellowship (funded by the Cancer Research Campaign) is available immediately and is renewable on an annual basis until September 1981.

Further details and application forms from the Academic Registrar, University of Warwick, Coventry CV4 7AL quoting Ref. No. 29/R/78. Closing date for receipt of applications is March 17, 1978. 1538(E)

IMPERIAL CANCER
RESEARCH FUND
RESEARCH FELLOWSHIP

Applications are invited for a Postdoctoral Research Fellow to work in area of surface antigens and receptors in normal and neoplastic epithelium with emphasis on application of S.E.M. marker probe procedures. Some experience in electron microscopy and immunochemistry or membrane biochemistry would be an advantage but not essential.

The appointment is tenable for two years with possible extension to three years and is available immediately.

Salary in range £3,678 to £4,698 (plus allowances) according to qualifications and experience.

Further information from Dr G. Hodges. Applications with curriculum vitae and names of two referees should be sent to The Secretary, Imperial Cancer Research Fund, Lincoln's Inn Fields, London WC2A 3PX by March 17, 1978. 1476(E)

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There are occasional opportunities for scientists to spend a period of a month or two in the *Nature* office working alongside our editorial staff.

We are at present inviting applicants for these positions for the coming year. Further details and application forms are available from The Editor, *Nature*, 4 Little Essex Street, London WC2. 1429(E)

Applications are invited for a

POSTDOCTORAL
RESEARCH FELLOWSHIP

for work on the mechanism of the photodynamic action of porphyrins in collaboration with Professor R. Bonnett and Professor I. A. Magnus. The position would suit an organic chemist or biochemist with an interest in photochemistry.

Initial salary in range £3,783 to £6,077 (including London Allowance). Please apply by letter, enclosing curriculum vitae and names and addresses of two referees, to Professor R. Bonnett, Department of Chemistry, Queen Mary College (University of London), Mile End Road, London E1 4NS. 1552(A)

RESEARCH FELLOWSHIP
Imperial Cancer Research Fund
Laboratories, Mill Hill, NW7

A postdoctoral Fellow is required to join a group working on the genetic and biochemical analysis of certain heat activated loci in *Drosophila melanogaster*, using cloned DNA fragments to investigate their organisation and expression.

Appointment will be for two years in the first instance with possible extension for a third year. Salary with entry according to qualifications and experience within range £4,649 to £5,669.

Further information from Dr D. Ish-Horowicz (01-959 3236). Applications with curriculum vitae and names of two referees, should be sent to The Secretary, Imperial Cancer Research Fund, Lincoln's Inn Fields, London WC2A 3PX by March 17, 1978. 1475(E)

THE UNIVERSITY OF
BRITISH COLUMBIA
DEPARTMENT OF
CHEMISTRY
GRADUATE TEACHING
ASSISTANTSHIPS

Graduate Teaching Assistantships are available for candidates with high academic records who wish to pursue further studies towards the M.Sc. and Ph.D. degrees in chemistry. Excellent research facilities are available in all areas of modern chemical science, ranging from bioinorganic chemistry to chemical physics. The minimum stipends including summer research assistantship will be \$6,120.00 per annum for first year and \$6,500.00 for students entering with a Master's Degree. The stipends are currently under review. In addition, many special merit awards are made to those Graduate Teaching Assistants who show excellence in the performance of their duties. Numerous graduate scholarships are also available for students with outstanding academic records.

Application forms and further particulars may be obtained from the undersigned:

Professor C. A. McDowell,
Department of Chemistry,
The University of
British Columbia,
Canada V6T 1W5

773(P)

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to be presented at and published in the Proceedings of the Second International Conference on Toxic Dinoflagellate Blooms, October 31–November 5, 1978, Key Biscayne, Florida. Sessions include Organisms, Environments, Toxins and Pharmacology. Data on environments of non-toxic forms will be considered. Abstracts must be received by April 15, 1978. Information and appropriate forms: Carrie Lewis, Bigelow Lab., W. Boothbay Harbor, Maine, U.S.A. 04575.

CALL FOR SAMPLES

of motile stages and resting cysts of dinoflagellates. Identifications given to those submitting samples and summarised at the Sample Preparation and Identification Workshop at the Second International Conference on Toxic Dinoflagellate Blooms. Sample collection, preservation, shipping deadlines / instructions: Carrie Lewis, Bigelow Lab., W. Boothbay Harbor, Maine, U.S.A. 04575. 1511(J)

Available by quotation: LOCUSTS, WORLD COCKROACHES, SNAILS (including African Achatina), WHITE-WORM, Edric Higginbottom, Laboratory Invertebrates, High Bradfield, Sheffield S6 6LJ. 1560(J)

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Further details may be obtained from the Administrative Secretary, Department of Physics, The University, Sheffield S3 7RH. 1508(D)

SYMPOSIUMS

SYMPOSIUM ON 'THEORY AND PRACTICE IN AFFINITY TECHNIQUES'. FOR INFORMATION SEE NATURE, PAGE XXIV, FEBRUARY 16, 1978. 1490(M)

VITELLOGENIN
WORKSHOP

to be held in BORGER (near GRONINGEN), The Netherlands, September 25, 26 and 27, 1978.

Sponsored by

European Molecular Biology
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and

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Fonger de Haan Fund,
The Netherlands.

The aim of the workshop is to discuss recent advances and future directions of research on the hormonal regulation of vitellogenin synthesis in vertebrates and insects.

There will be no registration fee and the costs of accommodation and meals will be covered by the organisers. Travel expenses can be partially reimbursed if necessary. Maximally 50 participants will be admitted and selection will be based on their present research interests.

Further information and application forms may be obtained from Professor Dr M. Gruber, Biochemisch Laboratorium, The University, Zernikelaan, Groningen, The Netherlands.

Closing date for applications:
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Under the sponsorship of the European Communities, the "Centro di Biologia Molecolare del Consiglio nazionale delle Ricerche (CNR)" is organising a interdisciplinary training course, to take place in Rome from September 18 to 30, 1978 on:

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 - I. Hemoglobin and isolated hemoglobin chains
 - (1) Interaction with ligands: equilibrium and kinetics (rapid mixing and relaxation techniques)
 - (2) Interaction with heterotropic allosteric effectors
 - (3) Abnormal hemoglobins.
 - II. Enzymes of the red blood cell involved in oxidative pathways and in the scavenging of high reactive oxygen derivatives.
 - III. The red blood cell membrane: structure, permeability, transport and membrane-associated proteins.
- B. The red blood cell as an integrated system.
 - (1) Oxygen binding by whole erythrocytes system
 - (2) Autoxidation phenomena
 - (3) Regulation of glucose metabolism
 - (4) Ageing and hemolysis.
- C. Hemoglobin biosynthesis and its regulation at the molecular level.

The working language of the course will be English.

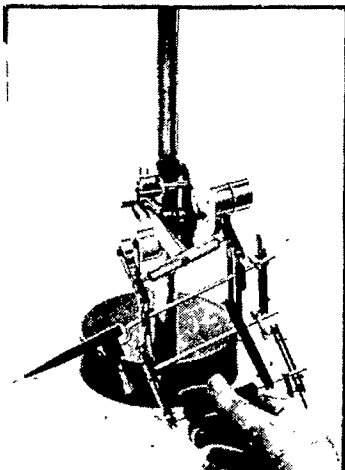
Applications for enrolment should reach the Molecular Biology and Radiobiology Course Secretariat, Commission of the European Communities DG XII, rue de la Loi 200, B-1049 Brussels, before 20 March 1978, and be accompanied by the applicant's personal history (stating knowledge of languages), a list of his scientific publications and a letter from the head of his laboratory.

Further information may be obtained from the same address.

1485(D)

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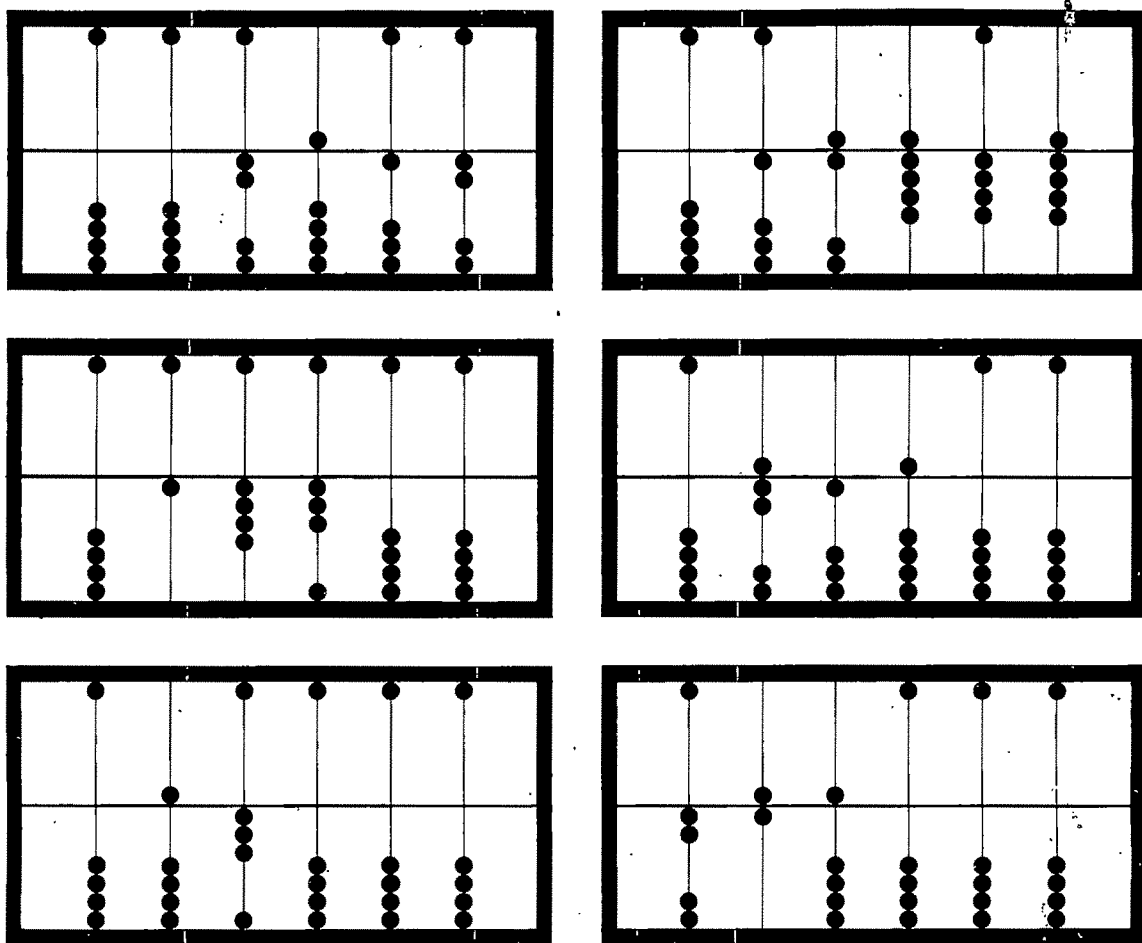
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